

# Targeted therapy and personalized medicine in hepatocellular carcinoma: drug resistance, mechanisms, and treatment strategies

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**Abstract:** Hepatocellular carcinoma (HCC) is characterized by a growing number of new cases diagnosed each year that is nearly equal to the number of deaths from this cancer. In a majority of the cases, HCC is associated with the underlying chronic liver disease, and it is diagnosed in advanced stage of disease when curative treatment options are not applicable. Sorafenib is a treatment of choice for patients with performance status 1 or 2 and/or macrovascular invasion or extrahepatic spread, and regorafenib is the only systemic treatment found to provide survival benefit in HCC patients progressing on sorafenib treatment. Other drugs tested in different trials failed to demonstrate any benefit. Disappointing results of numerous trials testing the efficacy of various drugs indicate that HCC has low sensitivity to chemotherapy that is in great part caused by multidrug resistance. Immunotherapy for HCC is a new challenging treatment option and involves immune checkpoint inhibitors/antibody-based therapy and peptide-based vaccines. Another challenging approach is microRNA-based therapy that involves two strategies. The first aims to inhibit oncogenic miRNAs by using miRNA antagonists and the second strategy is miRNA replacement, which involves the reintroduction of a tumor-suppressor miRNA mimetic to restore a loss of function.

**Keywords:** hepatocellular carcinoma, drug resistance, multimodal treatment, chemotherapy

## Introduction

Hepatocellular carcinoma (HCC) is characterized by a growing incidence worldwide.<sup>1,2</sup> It is a leading cause of cancer-related deaths, as it is the fifth most common cancer and the third most important cause of cancer-related death in men.<sup>2-5</sup> The growing number of new cases diagnosed each year is nearly equal to the number of deaths from this cancer, indicating that health care systems worldwide have no efficient answer to this deadly disease.

In some regions, HCC is the fastest rising cause of cancer-related death.<sup>6-10</sup> In spite of increased research and new treatment modalities, the overall prognosis for HCC patients remains poor.<sup>11</sup>

In a majority of the cases, HCC is associated with underlying chronic liver disease. Among different risk factors for the development of HCC, the two factors prevail: one is chronic hepatitis C virus infection and the other one is obesity and the metabolic syndrome.<sup>12-14</sup> These two factors are responsible for the growing incidence of HCC.<sup>12,14</sup> The epidemic of obesity is not only relevant for the USA but for Asia as well with a tendency to become the leading cause of the rise in HCC incidence.<sup>10,12</sup> Another important risk factor is chronic hepatitis B virus infection responsible for ~50% of all worldwide

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HCC cases.<sup>2,3,5</sup> Among 400 million people with hepatitis B virus (HBV) infection, ~25% will develop HCC.<sup>3,5,8,12</sup>

A majority of HCC cases are diagnosed in advanced stage of disease when curative treatment options (liver transplantation, liver resection, ablative procedures) cannot be applied. In principle, treatment allocation should be decided by a multidisciplinary tumor board for two reasons: the first one is to balance between different treatment modalities and the second is due to complexity of the disease (chronic liver disease associated with cancer). Appropriate patient selection and tailored treatment modality guided by personalized-based medicine should offer every HCC patient the best possible treatment outcome.<sup>15</sup>

In recent years, different staging systems for HCC have been developed, although none is universally accepted.<sup>16–20</sup> However, Barcelona Clinic Liver Cancer (BCLC) staging system endorsed by the American Association for the Study of the Liver (AASL) and European Association for the Study of the Liver (EASL), despite its limitations, has emerged as the most useful to guide HCC treatment.<sup>21,22</sup>

According to the BCLC staging system, chemotherapy is indicated for HCC patients with Eastern Cooperative Oncology Group (ECOG) performance status (PS) of 1 or 2 and/or macrovascular invasion or extrahepatic spread (BCLC stage C).<sup>21</sup> Based on the opinions of Asian experts, this category could be further stratified into a locally advanced stage without extrahepatic spread and an extrahepatic advanced stage.<sup>23,24</sup> This stratification has an impact on selecting the most optimal treatment approach.<sup>23</sup> Further stratification of patients in BCLC stage C according to liver function, or other prognostic variables, can be also explored.<sup>25,26</sup>

In the past, various conventional chemotherapy regimens were tested in numerous trials demonstrating disappointing results in the management of HCC.<sup>27</sup> Finally, the two milestone studies have positioned sorafenib, the first drug that demonstrated benefit in HCC patients, as a treatment of choice for BCLC stage C patients according to the EASL–European Organization for Research and Treatment of Cancer (EORTC) guidelines.<sup>21,28,29</sup>

However, the management of HCC patients in the BCLC C group should not be limited to sorafenib treatment only. The multimodal treatment of this patient population is feasible according to the treatment algorithm used in Asian countries.<sup>30–34</sup> The possible treatment modalities are transcatheter arterial chemoembolization (TACE),<sup>35</sup> hepatic arterial infusion chemotherapy (HAIC),<sup>36</sup> and radiotherapy (RT),<sup>37–40</sup> or combinations of locoregional therapies and sorafenib.<sup>41–43</sup> Drug resistance and toxicity limits the use of sorafenib or other drugs in the management of HCC patients.<sup>44</sup>

## Drug resistance

Disappointing results of numerous trials testing the efficacy of various drugs indicate that HCC has low sensitivity to chemotherapy. This is in great part caused by multidrug resistance (MDR).<sup>45</sup> Drug efflux pump, epithelial–mesenchymal transition (EMT), hypoxia-inducible factor1- $\alpha$  (Hif1- $\alpha$ ), and DNA damage repair are known mechanisms responsible for MDR.<sup>46–49</sup>

Several authors found that respective MDR mechanisms are closely related to intracellular calcium.<sup>50–53</sup> Wen et al explored the roles of intracellular calcium on various MDR-relevant mechanisms in HCC cells.<sup>54</sup> In their study, HCC cells were treated by stimulation with doxorubicin, hypoxia, and ionizing radiation, representing three models of MDR.<sup>54</sup> The authors found that the MDR-related mechanisms, EMT, Hif1- $\alpha$ -signaling, and DNA damage repair are all calcium dependent in HCC cells.<sup>54</sup>

Other authors studied the mechanisms of sorafenib resistance and found the important role of fibroblast growth factor 19 (FGF19).<sup>55–57</sup> *FGF19* is a metabolic regulator gene belonging to the hormone-like FGF family of signal molecules, and acts as an oncogenic driver in HCC.<sup>58–60</sup>

Gao et al found that *FGF19* is essential for sorafenib efficacy and resistance in the treatment of HCC.<sup>61</sup> The authors have demonstrated that elevated *FGF19* expression or hyperactivation of FGF19/FGFR4 signaling in HCC cells is one of the main mechanisms of sorafenib resistance.<sup>61</sup> In the same study, it was shown that blocking FGF19/FGFR4 axis by ponatinib, the third-generation tyrosine-kinase inhibitor, can overcome the resistance of HCC cells to sorafenib by enhancing reactive oxygen species-associated apoptosis.<sup>61</sup>

These and similar studies may provide the basis for developing treatment strategies to prevent single-drug resistance. Inhibition of FGF19/FGFR4 signaling is one of the possible strategies for overcoming sorafenib resistance in HCC.

## Molecular targeted therapy Sorafenib

Sorafenib is a molecular multikinase inhibitor of several tyrosine protein kinases (VEGFR and PDGFR); Raf kinases (C-Raf and B-Raf); and intracellular serine/threonine kinases (C-Raf, wild-type B-Raf, and mutant B-Raf)<sup>62–64</sup> (Table 1). This is the first molecular targeted agent that demonstrated survival benefit in nonresectable HCC patients.<sup>28,29</sup> Sorafenib induces autophagy which in turn suppresses tumor growth.<sup>65</sup>

The two milestone studies have established sorafenib, as a treatment of choice for HCC patients with ECOG PS of

**Table 1** Summary of sorafenib, tivantinib, and regorafenib mechanism of action, effect, and side effects

Drug	Mechanism	Effect	Side effects
Sorafenib	Multikinase inhibitor of: <ul style="list-style-type: none"> <li>– several tyrosine protein kinases (VEGFR and PDGFR)</li> <li>– Raf kinases (C-Raf and B-Raf)</li> <li>– intracellular serine/threonine kinases (C-Raf, wild-type B-Raf, and mutant B-Raf)</li> </ul>	Tumor growth suppression by autophagy	<ul style="list-style-type: none"> <li>– Gastrointestinal (diarrhea, increased lipase, increased amylase, nausea, anorexia, vomiting, and constipation)</li> <li>– Dermatologic (rash/desquamation, hand–foot skin reaction, alopecia, pruritus, and dry skin)</li> <li>– Cardiovascular (hypertension, angioedema, and congestive heart failure)</li> <li>– Hematologic (hypoalbuminemia, hemorrhage, anemia, and thrombocytopenia)</li> <li>– Nervous system (neuropathy and headache)</li> </ul>
Tivantinib	Highly selective inhibitor of c-MET receptor tyrosine kinase	<ul style="list-style-type: none"> <li>– Promotes apoptosis and cell growth arrest</li> <li>– Cytotoxic activity, even in cells that lack c-MET</li> <li>– Activation of cyclin B1 and inhibition of microtubule</li> </ul>	<ul style="list-style-type: none"> <li>– Hematologic toxicity (neutropenia, anemia, and leukopenia)</li> <li>– Fatigue, nausea, and vomiting</li> </ul>
Regorafenib	Multikinase inhibitor of VEGFR1-3, c-KIT, TIE-2, PDGFR- $\beta$ , FGFR-1, RET, c-RAF, BRAF, and p38 MAP kinase	Anti-angiogenic activity	Hand–foot skin reaction, diarrhea, fatigue, hypothyroidism, anorexia, hypertension, nausea, and voice changes

1 or 2 and/or macrovascular invasion or extrahepatic spread according to the EASL–EORTC guidelines.<sup>21,28,29</sup>

The findings of SHARP/Phase III trial conducted in the Western world have demonstrated prolonged median survival from 7.9 months (placebo group) to 10.7 months (sorafenib group) (hazard rate [HR]=0.69; 95% CI: 0.55–0.87;  $p=0.00058$ ).<sup>28</sup> Sorafenib also improved the time to radiological progression (from 2.8 months to 5.5 months).<sup>28</sup> The results of another Phase III trial, Asia-Pacific trial, have demonstrated a median overall survival of 6.5 months for a treatment group compared to 4.2 months for a placebo group (HR =0.68; 95% CI: 0.50–0.93;  $p=0.014$ )<sup>29</sup> (Table 2).

At the beginning, sorafenib was introduced as a well-tolerated drug. However, a subanalysis of the SHARP and Asia-Pacific trials and results of other studies have shown suboptimal tolerability of sorafenib; it was down-dosed in >50% patients and interrupted in 45% of patients due to severe adverse events (AEs) or compromised liver function.<sup>28,29,66–68</sup>

According to the results of several studies, the most important side effects are gastrointestinal (diarrhea 43%, increased lipase 41%, increased amylase 30%, nausea 23%, anorexia 16%, vomiting 16%, and constipation 15%), dermatologic (rash/desquamation 40%, hand–foot skin reaction 30%, alopecia 27%, pruritus 19%, and dry skin 11%), cardiovascular (hypertension 17%, angioedema, and congestive heart failure), hematologic (hypoalbuminemia 49%, hemorrhage 15%, anemia and thrombocytopenia), and nervous system side effects (neuropathy 13% and headache 10%).<sup>28,29,69–72</sup>

**Table 2** Summary of sorafenib, tivantinib, and regorafenib clinical outcomes

Drug	Clinical outcomes
Sorafenib	Prolonged median survival from 7.9 months (placebo group) to 10.7 months (sorafenib group) <sup>28</sup> Median overall survival of 6.5 months for sorafenib group compared to 4.2 months for a placebo group <sup>29</sup>
Tivantinib	Survival benefit in patients with advanced HCC who have failed or are intolerant to sorafenib
Regorafenib	Only systemic treatment found to provide survival benefit in HCC patients progressing on sorafenib treatment <sup>106</sup>

**Abbreviation:** HCC, hepatocellular carcinoma.

The safety of sorafenib treatment in real-life conditions was evaluated in Phase IV, GIDEON trial; the second interim analysis from 2011 showed a median survival of 10.3 months for Child A patients and 4.8 months for Child B patients and AE rate comparable to the two leading studies.<sup>73</sup>

Further studies on sorafenib and other drugs demonstrated disappointing results.<sup>74</sup> Phase III trials based on head-to-head comparison between sorafenib and other drugs (sunitinib, brivanib, erlotinib) failed to demonstrate any benefit.<sup>74</sup> The same results were found in Phase III trials on second-line therapies comparing new biological agents (everolimus, brivanib, and ramucirumab) versus placebo.<sup>74</sup>

The combination regimen of sorafenib and chemotherapeutic agent (“sorafenib plus concept”) was assessed in several Phase II/III trials; however, the combination failed to show superiority in clinical trials.<sup>75,76</sup>

The impact of viral status on survival in HCC patients receiving sorafenib was analyzed by Jackson et al.<sup>77</sup> They conducted a meta-analysis of three large prospective randomized trials (total of 3,256 patients) in which sorafenib was the control arm.<sup>77</sup> They found an improved overall survival in patients who were hepatitis C virus (HCV) positive and HBV negative and no evidence of any improvement in overall survival attributable to sorafenib in patients who were HBV positive and HCV negative.<sup>77</sup> The same results were found in subgroup analysis of the SHARP trial demonstrating the superior median overall survival in HCV-positive patients.<sup>78</sup>

In the same study, the subgroup of alcohol-related HCC patients was analyzed.<sup>78</sup> The sorafenib group was associated with a longer median overall survival (10.3 vs 8.0 months) and time to progression (5.5 vs 3.9 months) and a higher disease control rate (54.4% vs 38.8%) than placebo.<sup>78</sup> Although in alcoholics the surveillance for HCC could be less effective because of the socioeconomic context, the stage at the diagnosis and the prognosis are not different compared to viral HCCs.<sup>79</sup>

Nonalcoholic steatohepatitis (NASH)-associated HCC patients are another important subset of patients because of the increasing incidence of NASH worldwide.<sup>8,10,12</sup> Weinmann et al have analyzed a large cohort of HCC patients (1,119) treated in an 11-year period and compared the outcome of NASH-HCC and non-NASH-HCC patients managed by sorafenib.<sup>80</sup> Systemic therapy with sorafenib as a first-line treatment was significantly more frequently performed in NASH-HCC (17.8% vs 3.5%,  $p < 0.001$ ). The overall survival in NASH-HCC was 4.22 months shorter compared to non-NASH-HCC (median 11.28 vs 15.5 months).<sup>80</sup> However, in the absence of cirrhosis, NASH-HCC patients demonstrated a trend to an increased overall survival compared to non-NASH-HCC patients (43.4 vs 25 months,  $p = 0.748$ ).<sup>80</sup>

The safety and efficacy of sorafenib in HCC patients with diabetes was confirmed by Di Constanzo et al.<sup>81</sup> They found a comparable frequency of main AEs in both diabetes and nondiabetes groups, with the exception of rash, which was more frequent among diabetes patients (27.5% vs 17.6%,  $p = 0.047$ ). The median overall survival was 9 months in nondiabetes and 10 months in the diabetes group ( $p = 0.535$ ).<sup>81</sup> Median time to progression was longer in diabetes than in the nondiabetes group ( $p = 0.038$ ), suggesting a better anticancer effect of sorafenib in patients with diabetes.<sup>81</sup> However, the concomitant use of sorafenib and metformin was associated with shorter median progression-free survival compared to patients receiving sorafenib alone (2.6 vs 5.0 months,  $p = 0.029$ ).<sup>82</sup> The median overall survival of patients treated with the combination was 10.4 months compared to

15.1 months for patients who were not given metformin therapy ( $p = 0.014$ ). These results suggest possible resistance to sorafenib in metformin-treated patients.<sup>82</sup>

The possible use of sorafenib in adjuvant settings was assessed in the STORM trial. The trial enrolled 1,114 patients randomized to take either sorafenib or placebo.<sup>83</sup> However, the study did not meet its primary and secondary endpoints since no differences were observed regarding recurrence-free survival (33.4 vs 33.8 months; HR = 0.94; 95% CI: 0.78–1.13,  $p = 0.26$ ), time to recurrence (38.6 vs 35.8 months; HR = 0.89, 95% CI: 0.73–1.08), and overall survival (not reached vs not reached, HR = 0.99, 95% CI: 0.76–1.30,  $p = 0.48$ ).<sup>83</sup>

The combination of sorafenib and transarterial chemoembolization for HCC patients in intermediate stage (BCLC B group) was evaluated in the SPACE trial; however, the primary end point was not reached.<sup>84</sup> Among 307 patients randomized, 154 received sorafenib and 153 received placebo. Median time to progression for patients receiving sorafenib plus drug-eluting bead (DEB)-TACE or placebo plus DEB-TACE was similar (169 vs 166 days, respectively; HR = 0.797,  $p = 0.072$ ).<sup>84</sup>

## Tivantinib

Tivantinib (ARQ 197) is a small, oral, highly selective inhibitor of c-MET receptor tyrosine kinase<sup>85</sup> (Table 1). The MET encodes for cellular mesenchymal-epithelial transition factor (c-MET).<sup>86</sup> Tivantinib inhibits in a non-ATP-competitive manner MET autoactivation by selectively targeting the inactive nonphosphorylated form of the kinase.<sup>87,88</sup> The inhibition of c-MET by tivantinib promotes apoptosis and cell growth arrest.<sup>89–91</sup> Recent studies have shown that tivantinib possesses cytotoxic activity, even in cells that lack c-MET.<sup>90–94</sup> Also, some studies hypothesize that tivantinib can activate cyclin B1<sup>95</sup> and inhibit microtubule.<sup>90,94</sup> Several preclinical and clinical studies showed that tivantinib can act as a single agent<sup>85,96</sup> and in combination with sorafenib.<sup>97–99</sup>

In Phase I and II trials, tivantinib has shown a survival benefit in patients with advanced HCC who have failed or are intolerant to sorafenib.<sup>100–102</sup> Twenty-one patients were enrolled to a 28-day treatment protocol. The tumor response was evaluated only in 16 patients, but tumor regression was not recorded. The median disease progression interval was 3.3 months (range 1.47–5.3 months)<sup>97</sup> (Table 2).

Those patients who have tumors with high expression of MET (MET-high) appear to derive the greatest benefit from tivantinib therapy. Currently, two large randomized double-blind placebo-controlled Phase III trials METIV-HCC trial [NCT01755767] and JET-HCC trial [NCT02029157]) are evaluating tivantinib in patients with MET-high advanced HCC.<sup>103,104</sup>

## Regorafenib

Regorafenib is a multikinase inhibitor of VEGFR1-3, c-KIT, TIE-2, PDGFR- $\beta$ , FGFR-1, RET, c-RAF, BRAF, and p38 MAP kinase<sup>105</sup> (Table1). Its structure is similar to sorafenib; however, higher potency is expected due to the addition of a fluorine atom in the central phenyl ring.<sup>106</sup>

In the multicenter analysis of treatment pattern and outcomes in Hong Kong, 45 consecutive patients with metastatic colorectal cancer treated with regorafenib after failure of all standard systemic options were analyzed.<sup>107</sup> Initial dose was 160 mg in the group of 20 patients, while the remaining patients received lower doses. Of 31 patients, one had partial response and ten had stable disease.<sup>107</sup> The most common hematological AE was anemia (8.9%), and nonhematological AE was hand-foot skin reaction (26.7%).<sup>107</sup>

Efficacy and safety of regorafenib has been also confirmed for treatment of gastrointestinal stromal tumor after progression or toxicity during imatinib and sunitinib treatment.<sup>108,109</sup>

Safety of regorafenib as second-line therapy for intermediate or advanced HCC was assessed in the multicenter, Phase II study, by Bruix et al.<sup>110</sup> Thirty-six patients were enrolled and received 160 mg dose once daily during 3 weeks on and 1 week off treatment. Treatment was interrupted when disease progression (n=10), unacceptable toxicity (n=20), or death (n=1) occurred. Treatment-related AEs were: hand-foot skin reaction, diarrhea, fatigue, hypothyroidism, anorexia, hypertension, nausea, and voice changes. Partial response was achieved in one patient and stable disease in 25. Median time to progression and overall survival were 4.3 and 13.8 months, respectively<sup>110</sup> (Table 2).

A larger, international, multicentric Phase III trial (RESORCE) concluded that regorafenib is the only systemic treatment found to provide survival benefit in HCC patients progressing on sorafenib treatment.<sup>111</sup> During the study period, 573 patients were randomized (379 to regorafenib and 194 to placebo). Regorafenib group experienced longer median survival compared to placebo group (10.6 vs 7.8 months).<sup>111</sup> The most common grade 3 or 4 treatment-related AEs were hypertension, hand-foot skin reaction, fatigue, and diarrhea, similar to sorafenib AE profile. Mortality rate was 13% in the regorafenib group and 20% in the placebo group. Seven deaths (2%) were associated with study drug treatment and two (1%) in the placebo group were related with liver failure.<sup>111</sup>

The approval of regorafenib for this indication is expected during 2017.<sup>106</sup>

## Immunotherapy for HCC

### Immune checkpoint inhibitors – antibody-based therapy

The main goal of previous cancer immunotherapy has been to enhance immune cell activity to kill the cancer cells. However, this does not result in actual activation of the immune system because of the inhibition signal by checkpoint molecules.

Under physiologic conditions, the liver is an organ of relative immune tolerance, and checkpoint pathways play a key role in this process. This function is very significant as the liver is persistently exposed to food-borne and microbial antigens delivered from the gut. HCC immune tolerance is mediated through decreased co-stimulatory or increased inhibitory checkpoint signaling that leads into immunosuppression. Human cytotoxic T-lymphocyte antigen 4 (CTLA-4) and a programmed death 1 (PD-1) and its ligand (PD-L1) are among the several inhibitory checkpoints that have been extensively investigated, as well as related with defective immune process in HCC. CTLA-4 is expressed constitutively on regulatory T cells (Tregs) and transiently on a broad range of T cells at the early activation stage. In the anticancer immunity, CTLA-4 can inhibit the activation and proliferation of T cells that recognize cancer antigens.<sup>112</sup>

PD-1 is an immune coinhibitory receptor expressed on T cells, B cells, NK cells, and myeloid cells. On T cells, PD-1 suppresses antigen-specific T-cell activation through interactions with its ligands PD-L1 and PD-L2.<sup>113–115</sup> PD-L1 is expressed on hepatocytes,<sup>116</sup> Kupffer cells,<sup>117</sup> and liver sinusoidal endothelial cells.<sup>118–120</sup> Currently, there are several immune checkpoint monoclonal antibody blockers, such as ipilimumab, tremelimumab, nivolumab, MED14736, anti-LAG-3 (BMS-986016), and pembrolizumab.

Several studies show that higher levels of circulating PD-1+/CD8+ T cells correlate with disease progression;<sup>121</sup> as well, increased PD-L1 levels in HCC patients were associated with poorer prognosis.<sup>122</sup>

Less data from preclinical and translational studies exist regarding the mechanistic role of CTLA-4 in HCC.<sup>123</sup> It is known that high levels of CTLA-4 expression on hepatic dendritic cells have been linked with inhibition of T-cell proliferation and T-cell apoptosis in HCC,<sup>124</sup> while high levels of CTLA-4 expression by Tregs correlate with decreased production of cytotoxic enzymes by effector T cells in HCC.<sup>113</sup>

Several clinical trials of immune checkpoint inhibitors in HCC are ongoing. Sangro et al in a Phase II trial (ClinicalTrials.gov: NCT01008358) evaluated the efficacy and safety of



anti-CTLA-4 antibodies (tremelimumab) for the treatment of HCC.<sup>120</sup> Patients with unresectable HCC and chronic hepatitis C received tremelimumab as a monotherapy. Treatment was generally well tolerated. The patients most frequently developed skin rash, fatigue, and anorexia. Severe immune-mediated AEs were not recorded. Among treated patients, there was a 76.4% disease control rate and 17.6% partial response. The median time to progression was 6.5 months.<sup>125</sup>

Regarding the PD-1 therapies, in a Phase I/II trial (MEDI4736), a fully human anti-PD-L1 monoclonal antibody is being evaluated in multiple advanced cancers, including HCC (ClinicalTrials.gov: NCT01938612).<sup>126</sup> Until now, only a relatively favorable safety profile has been reported. Preliminary results of a Phase I/II trial of nivolumab, a fully human IgG4 anti-PD-1 monoclonal antibody, were reported (ClinicalTrials.gov: NCT01658878).<sup>127</sup>

This study included eleven patients with HCV and ten patients with HBV infection, while 50% of patients were unaffected. Approximately 19% of patients responded to nivolumab, two patients achieved complete response, while six patients had partial response. Overall survival was 72% at 6 months and 62% at 12 months. Of note, 68% of patients had received prior sorafenib therapy, and responses were seen in both patients with and without viral hepatitis. AEs that included transaminase and lipase elevations along with anemia and fatigue were observed in 19% of patients.<sup>127</sup>

Immune checkpoint inhibition can induce immune-related AEs such as hyperthyroidism, hypothyroidism, Type 1 diabetes mellitus, myasthenia gravis, rheumatoid arthritis, or Addison's disease, along with dry mouth, hepatitis, dermatopathy, pancreatitis, pneumonitis, adrenal insufficiency, and enteritis. Many of these AEs can be controlled by withdrawal of immune checkpoint inhibitors and initiation of steroid therapy. Immune-related AEs are least frequent in patients treated with anti-PD-L1 antibodies and most frequent in patients treated with anti-CTLA-4 antibodies.<sup>128</sup>

Having in mind multiple mechanisms by which HCC evades the immune system, in addition to anti-CTLA-4 and PD-1 monotherapy, combination therapy may be necessary to achieve better therapeutic outcomes than the corresponding monotherapies. In November 2015, a Phase I/II study (ClinicalTrials.gov: NCT01658878) was modified and included a nivolumab/ipilimumab combination arm.<sup>127</sup> One other trial of tremelimumab with MEDI4736 (a PD-L1 antagonist) for patients with unresectable HCC (ClinicalTrials.gov: NCT02519348) is ongoing. Also, the combination of checkpoint inhibitor with sorafenib appears promising, as sorafenib exerts immunomodulatory effect.<sup>129</sup>

## Peptide vaccines

Another approach to immunotherapy involves peptide-based vaccines. GPC3 belongs to the glypican family of heparan sulfate proteoglycans, which are linked to the outer surface of the cell membrane through a glycosylphosphatidylinositol anchor.<sup>130</sup> Since it is specifically overexpressed in HCC (72%–81%), GPC3 is an ideal target for antigen-specific immunotherapy against HCC.<sup>131,132</sup>

A nonrandomized, open-label, Phase I clinical trial of a GPC3-derived peptide vaccine for advanced HCC patients was conducted in Japan.<sup>133</sup> In this trial, 33 patients with advanced HCC received peptide vaccines. This trial demonstrated that the vaccine was well tolerated, and for the first time this study showed that the frequency of GPC3-derived, peptide-specific cytotoxic T lymphocytes (CTLs) was correlated with overall survival in patients with HCC receiving a peptide vaccine.<sup>134</sup>

The same group conducted a Phase II clinical trial of the GPC3-derived peptide vaccine in the adjuvant setting (UMIN-CTR: 000002614).<sup>134</sup> Forty-one patients with initial HCC who had undergone surgery or radiofrequency ablation were enrolled in this Phase II, open-label, single-arm trial. Sawada et al investigated case-control subjects, where selected patients treated surgically during the same period were analyzed.<sup>134</sup> Six patients received radiofrequency ablation therapy while 35 received surgery. The recurrence rate tended to be lower in the 35 patients treated with surgery plus vaccination compared to 33 patients who underwent surgery alone. Twenty-five patients treated with surgery and vaccination had GPC3-positive tumors; the recurrence rate in this group was significantly lower compared to that in 21 GPC3-positive patients who received surgery only.<sup>134</sup> The GPC3 peptide vaccine improved the 1-year recurrence rate in patients with GPC3-positive tumors.

## MicroRNA-based therapies

Nucleic acid-based drugs such as microRNAs (miRNAs) have promising therapeutic potential for HCC treatment. miRNAs are an abundant class of endogenous, short (20–25 nucleotides in length) non-coding RNAs. miRNAs control the stability and translation of targeted messenger RNAs (mRNAs) through complementary interaction with the 3' untranslated region of target genes (or of complementary sequences).

miRNAs are involved in fundamental cellular processes, like embryonic development, differentiation, cell cycle, metabolism, and in carcinogenesis and tumor progression.<sup>135–139</sup> Accumulating evidence has addressed that

miRNAs are involved in oncogene and tumor-suppressor pathways.<sup>140–143</sup>

Several studies have shown that expression of miRNA is deregulated in HCC in comparison with normal liver tissue. Thus, for example, miRNAs are both upregulated (miR-21, miR-221, miR-222, miR-224, and miR-17-92) and down-regulated (miR-29, miR-122, miR-200, miR-123, miR-199a, and miR-199b, let-7) in association with HCC.<sup>144,145</sup>

Cellular miRNA, like miR-196, plays a protective role in HCV-induced HCC by upregulating hemoxygenase 1 (HMOX1) expression and inhibiting HCV transcription.<sup>146</sup> On the other hand, miR-217 could promote ethanol-induced fat accumulation in hepatocytes,<sup>147</sup> while miR-126 was found decreased in alcohol-related HCC.<sup>148</sup> miR-21 and miR-155 are also involved in the pathogenesis of NASH. Results from some studies showed a correlation between modulation of cellular miRNAs abundance and the sensitivity of HCC cancer cells to chemotherapeutic drugs. Thus, for example, overexpression and restoration of miR-122 increases the sensitivity of HCC cells to chemotherapeutic agents, such as doxorubicin or sorafenib.<sup>149,150</sup>

The therapeutic application of miRNAs involves two strategies. The first aims to inhibit oncogenic miRNAs by using miRNA antagonists, such as antimiRs, or antagoniRs.<sup>151</sup> miRNA antagonists are single-stranded RNA molecules, which act through complementary base-pairing with miRNAs. The second strategy, miRNA replacement, involves the reintroduction of a tumor-suppressor miRNA mimetic to restore a loss of function.<sup>152</sup>

However, two main problems for miRNA-based therapeutics are off-target effects, since one miRNA can target multiple genes and pathways, and their delivery. One possibility to overcome these is usage of the adeno-associated viral vectors with lower risk of vector-related toxicities and good gene transfer efficacy.

Kota et al administered miR-26a, which is frequently downregulated in HCC and acts as tumor suppressor, in a mouse model using adeno-associated virus. They found that ectopic expression of miR-26a results in inhibition of cancer cell proliferation and induction of tumor-specific apoptosis.<sup>153</sup> Also, a Phase I trial which investigates the role of MRX34, a liposome-based miR-34 mimic, is currently ongoing.<sup>154</sup>

## Conclusion

The rising incidence of HCC occurrence in patients with underlying chronic liver disease, initial presentation in advanced stage in the majority of cases, and high mortality rates demonstrate an urgent need to develop effective treatment

and prevention strategies for this complex disease. Although multimodal management is clearly needed for the majority of HCC patients, appropriate randomized clinical trials or meta-analyses addressing this problem are lacking. The consensus for successful management of advanced HCC patients using a multimodal approach should be further established.

Today, sorafenib is the first-line and regorafenib is the second-line therapy for HCC patients with PS 1 or 2 and/or macrovascular invasion or extrahepatic spread, providing a median gain in overall survival of <3 months. The genetic instability makes HCC commonly resistant to chemotherapy and additional management strategies are needed.

The introduction of checkpoint regulators has opened a new horizon for cancer immunotherapy. As indicated by several trials, immunotherapeutics are very promising therapeutic tools for HCC treatment. In future, HCC immunotherapy should be rationally combined with other treatment modalities like surgery, thermal ablation, and cytotoxic agents to maximize its therapeutic efficacy.

Therapeutic interventions are challenging for this disease, although the majority of them are providing palliative treatment only. In the upcoming months, data of several ongoing first-line and second-line trials will become available and might further change the care of patients with advanced HCC.

## Disclosure

The authors report no conflicts of interest in this work.

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