Review article: systemic treatment of hepatocellular carcinoma

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Summary

Background: The approval of the tyrosine kinase inhibitor sorafenib in 2007 marked a milestone in the treatment of hepatocellular carcinoma, as sorafenib was the first systemic therapy to show a survival benefit in patients with advanced hepatocellular carcinoma. Since then many drugs failed in the first- and second-line setting and it took almost another decade until further tyrosine kinase inhibitors succeeded in phase III trials.

Aim: To summarise the evolving field of systemic therapy of hepatocellular carcinoma.

Methods: We reviewed recently published studies identified from PubMed and data presented at recent meetings. Main search terms included hepatocellular carcinoma, tyrosine kinase inhibitors, immunotherapy, immune checkpoint inhibitors, sorafenib, regorafenib, lenvatinib, cabozantinib, ramucirumab, and nivolumab.

Results: We discuss the evolution of targeted therapies since the approval of sorafenib including failures and recent advances. We also elaborate the unmet need of biomarkers to guide treatment decisions and discuss the emerging field of immunotherapy in hepatocellular carcinoma.

Conclusions: The tyrosine kinase inhibitors sorafenib (first line) and regorafenib (second line) have been approved for hepatocellular carcinoma, and the immune checkpoint inhibitor nivolumab obtained conditional approval for sorafenib-experienced patients in the United States. With lenvatinib in the first line, and cabozantinib and ramucirumab in sorafenib-experienced patients, three more targeted therapies reached their primary endpoint in phase III trials and may soon be added to the treatment armamentarium.
1 | INTRODUCTION

Hepatocellular carcinoma (HCC) is the most common primary liver cancer,1 usually develops in patients with liver cirrhosis,2,3 and represents the second most common cause of cancer-related death.4 Potential curative therapies include resection, liver transplantation, and local ablative therapies, but these are reserved for early stages, characterised by small tumours limited to the liver. Transarterial chemoembolization (TACE) and systemic therapies are the only available treatment options in the palliative setting,5 while transarterial radioembolisation is struggling (and currently unable) to find a place in the evidence-based treatment landscape.6-8 Patients with either symptomatic disease (performance status 1-2), macrovascular tumour invasion, or extrahepatic metastases (advanced stage HCC) are classical candidates for systemic treatment according to current guidelines.5 Patients with multifocal HCC and compensated liver disease (intermediate stage HCC) should be treated with TACE.5 However, these patients may become candidates for systemic therapies under certain circumstances (Figure 1): if they fail to respond to TACE (failure of 2 rounds of TACE), develop untreatable progression (major intrahepatic progression, macrovascular invasion, metastasis, symptomatic progression), show deterioration of liver function (ascites, decompensation),5,10 or show unfavourable disease characteristics at baseline (Hepatoma arterial-embolisation prognostic score C or D; Barcelona-Clinic Liver Cancer B subclass 3 or 4; STATE score <18).11-13 Liver function in particular should be monitored cautiously as even discrete subclinical worsening of liver function is associated with poorer outcome after repeated TACE.14,15

To date, only tyrosine kinase inhibitors have been approved globally and additionally an immune checkpoint inhibitor in the United States only.16 Conventional chemotherapy is not recommended in HCC due to lack of efficacy,5 and only recently two phase III studies testing chemotherapy in advanced HCC again failed.17,18 Whether a subset of patients with HCC may have some benefit from chemotherapy or not is currently terminated due to lack of enrolment. Thus, prospective data on sorafenib in Child-Pugh stage C are unlikely to derive a clinical meaningful benefit from any systemic therapy and should just receive best supportive care, if not a candidate for liver transplantation.5 A phase III trial (BOOST, NCT01405573) investigating if Child-Pugh B patients may benefit from sorafenib treatment was unfortunately terminated due to lack of enrolment. Thus, prospective data on sorafenib in Child-Pugh B patients are still missing, hindering clear recommendations for this heterogeneous subgroup of patients.

This review focuses on the evolution of targeted therapies since the approval of sorafenib, including failures and recent advances, and discusses the unmet need of biomarker-driven treatment strategies as well as the emerging field of immunotherapy in HCC.

2 | TARGETED THERAPIES

2.1 | Sorafenib

For the longest time no effective drug treatment was available for patients with HCC until the unprecedented success of the multitargeting kinase inhibitor sorafenib. The approval in 2007 was based on the positive results of the well-known SHARP study, a randomised controlled phase III trial, which showed a significant prolongation of median overall survival (OS) for sorafenib vs placebo (10.7 vs 7.9 months; hazard ratio (HR), 0.69), reflecting an extension of OS by 44%.20 A second phase III trial conducted in the Asia-Pacific region showed similar results regarding HR, even though patients in both groups had a shorter absolute OS compared to patients in the SHARP trial (Table 1). This was probably because of the enrolment of patients with more advanced disease in terms of extrahepatic spread, number of intrahepatic lesions, alpha-Fetoprotein (AFP), and performance status.21 Hence, sorafenib showed a survival benefit in two independent phase III trials conducted in different geographic regions, and became the standard of care for advanced HCC.3,5 Side effects were mostly mild to moderate and manageable, with diarrhoea and dermatological side effects (eg hand-foot-skin reaction, rash, pruritus) being the most frequent and troublesome adverse events.20,21 The occurrence of dermatological events was later shown to be associated with a better outcome.22,23

The efficacy of sorafenib is likely a result of the fine balance between its antitumour effects and the mild and manageable toxicity profile.24 A potential beneficial effect of sorafenib on the portal hypertensive syndrome, which may contribute to the improved survival in HCC patients, was reported in experimental models25 and small clinical pilot studies26,27 but not yet confirmed in large prospective trials.

Notably, both phase III trials included only patients with well-preserved liver function, a common practice in HCC studies to avoid a potential masking of a drug-induced antitumour effect by death from underlying liver disease.2,28 Data from our real-life cohort29 and that of others30 showed that the Child-Pugh score was a strong predictor for OS (median OS for Child-Pugh A/B/C, 11.3/5.5/1.6 months29). These data were confirmed by the GIDEON study, a large global prospective noninterventional phase IV observational study.31 Consequently, patients with Child-Pugh stage C are unlikely to derive a clinical meaningful benefit from any systemic therapy and should just receive best supportive care, if not a candidate for liver transplantation.5 A phase III trial (BOOST, NCT01405573) investigating if Child-Pugh B patients may benefit from sorafenib treatment was unfortunately terminated due to lack of enrolment. Thus, prospective data on sorafenib in Child-Pugh B patients are still missing, hindering clear recommendations for this heterogeneous subgroup of patients.

Given the success of sorafenib in advanced HCC, sorafenib was later on also evaluated in patients with early and intermediate stage tumours. A phase III randomised controlled trial (STORM) investigated sorafenib in the adjuvant setting after curative resection or local ablation. However, the study failed to reach its primary end-point recurrence-free survival.32 A phase II randomised controlled trial (SPACE) investigated the combination of drug-eluting-beads-TACE plus sorafenib or placebo in patients with intermediate HCC.33 Sorafenib failed to prolong the primary endpoint time to recurrence (TTP) in a clinically relevant manner (sorafenib vs placebo, 169 vs 166 days), partly also due to shortcomings in trial design. As a result of strict TACE discontinuation criteria, more than one-third of patients in the sorafenib group received only one TACE procedure, but at least 30% of these patients received further TACE cycles outside the study. This may have negatively affected the outcome of this study.33 Two phase III studies testing this combination also failed to show an improved outcome for sorafenib.34,35 Results of a Japanese phase II study (TACTICS) evaluating conventional lipiodol TACE plus sorafenib vs TACE
alone in HCC without macrovascular invasion/extrahepatic metastases were recently presented. The combination significantly prolonged the primary endpoint progression-free survival (PFS) vs TACE alone (25.2 vs 13.5 months; HR 0.59; \( P = 0.006 \)). Possible reasons for the success of this trial are the longer exposure to sorafenib compared to other trials, and the fact that new, previously untreated lesions were not regarded as progressive disease/treatment failure prompting discontinuation of TACE. These positive results may revive the discussion on this almost abandoned strategy in intermediate stage HCC.

Taken together, sorafenib was the first systemic therapy that showed a moderate but significant survival benefit and consequently became the first drug to be approved for HCC. Sorafenib is recommended for patients with advanced stage HCC or those who progressed on TACE. As data on Child-Pugh B patients are still lacking, clear recommendations for its use can only be made for patients with Child-Pugh class A. Large studies failed to show a benefit of sorafenib in the adjuvant setting or in combination with loco-regional therapies.

### 2.2 Failed phase III trials

The success of sorafenib has increased the interest to develop drugs in HCC. But instead of seeing more effective drugs being added to
the therapeutic armamentarium, we had to witness several compounds failing in randomised controlled phase III trials, both in the first-line (sunitinib, brivanib, linifanib, erlotinib57-60) and second-line (brivanib, everolimus, ramucirumab61-63) settings (Figure 2).

Potential reasons for failure have been discussed elsewhere, and include high toxicity (sunitinib64), modest efficacy (erlotinib, linifanib, brivanib, everolimus57,59,60,62), non-inferiority design with a small window of opportunity (linifanib, brivanib57,59), and a clinical imbalance between the test and placebo arm (brivanib61).

Notably, most drugs proceeded to phase III based on efficacy data obtained from small single-arm phase II studies, often using surrogate endpoints such as radiological response, TTP, or PFS. These surrogate endpoints are only poor predictors of OS in HCC, where even a significant response often does not translate into a survival benefit61,62. Additionally, composite endpoints (e.g., PFS) are vulnerable, as death from underlying cirrhosis may mask potential drug effects, and are generally discouraged as primary endpoints in HCC trials28. It was recently recommended that drugs should be tested in properly powered phase II studies with a control arm or at least with a large enough sample size in order to minimise a potential selection bias and random errors; the use of OS as a primary endpoint should also be considered in these trials.24 However, with more drugs becoming available in the second- and third-line setting influencing the outcome of HCC patients, OS may not adequately reflect the effect of a tested first-line treatment, making future trial design even more difficult.

Moreover, the pattern of progression during sorafenib therapy influences postprogression survival and could therefore affect the results of second-line trials.44 Given that the failed second-line phase III trials were designed before the publication of these important findings, none of the studies stratified patients according to the pattern of progression. Thus, it could well be that an enrichment of the worse progression pattern (new extrahepatic lesion/macrovascular invasion) may have occurred in the test arm, masking a potential drug effect.44,45

Finally, since HCC can be diagnosed by radiology alone in patients with liver cirrhosis, a tumour biopsy, allowing tissue-based biomarker analysis to potentially rescue a drug at least in a subset of patients, was not mandatory in most phase III trials.24

2.3 Recent advancements

After the approval of sorafenib, it took almost a decade until another drug could succeed in phase III, and again it was a multityrosine kinase inhibitor tested in an unselected “all-comer” cohort. Regorafenib was evaluated in a second-line phase III trial (RESORCE) and demonstrated a significant survival benefit compared to placebo (median OS, 10.6 vs 7.8 months; HR 0.63).46 Similar to the phase III trials of sorafenib,20,21 only patients with Child-Pugh stage A were allowed in order to minimise a potential confounding effect of advanced liver cirrhosis on OS. Because of the similar toxicity profile of both drugs, patients intolerant to sorafenib were excluded.46 Hence, no conclusions can be made about the efficacy of regorafenib in patients intolerant to sorafenib and in those with more advanced liver dysfunction (Child-Pugh B).46 The most common adverse events were mostly of mild grade and included hand-foot-skin reaction, fatigue, and diarrhoea. However, including only patients who tolerated sorafenib could have reduced the occurrence of severe side effects.46 As previously shown for sorafenib,22 development of hand-foot-skin reaction was associated with improved survival with regorafenib in an exploratory retrospective analysis.47

Unlike in the negative second-line study of brivanib,43 stratification was done separately for macrovascular invasion and extrahepatic spread and therefore allowed both prognostic variables to be well balanced between the groups. Even though not a stratification factor, the pattern of progression was equally distributed between both arms,46 which may have contributed to the positive outcome of this study. Regorafenib was finally approved for the indication “HCC” in 2017 in Europe and the United States.

Based on promising results of a phase II study, the multityrosine kinase inhibitor lenvatinib was tested against sorafenib in a phase III trial with the primary endpoint being non-inferiority in OS (non-inferiority margin: upper limit of the two-sided 95% confidence interval for HR below 1.08).49 Only patients with Child-Pugh stage A were included and patients with extensive tumour load (≥50% of the liver), bile duct invasion, or invasion of the main portal vein were excluded. Dosing of lenvatinib was based on body weight (8 mg
<60 kg and 12 mg ≥60 kg once daily, as lenvatinib exposure was influenced by body weight in the phase II study.\textsuperscript{48}

The study finally reached its primary endpoint with a HR of 0.92 (95% CI, 0.79-1.06) and a median OS of 13.6 months for lenvatinib and 12.3 months for sorafenib.\textsuperscript{49} Forest plots for OS revealed that lenvatinib was most effective compared to sorafenib in patients with baseline AFP ≥200 ng/mL (HR, 0.78; 95% CI, 0.63-0.98) and least effective in patients without macrovascular invasion/extrahepatic spread and those from the Western region. The latter is of special note since about two-thirds of the study population came from the Asia-Pacific region and only one-third from Western countries. Secondary endpoints (PFS, TTP, objective response rate) were significantly better with lenvatinib. This observation underlines again that these surrogate endpoints only poorly predict OS in HCC. The rates of treatment-related emergent adverse events ≥3 (57% vs 49%) and treatment-related serious treatment-emergent adverse events (18% vs 10%) were higher with lenvatinib. Arterial hypertension was the most common adverse event with lenvatinib occurring more often in the lenvatinib arm (42% vs 30%). The frequency of skin-related treatment was the most common side effect in the sorafenib arm—was higher with sorafenib (52% vs 27%). Notably, as safety was not a predefined study endpoint, both drugs cannot be compared reliably. Health-related quality of life scores for role functioning, pain, diarrhoea, nutrition, and body image worsened earlier with sorafenib.\textsuperscript{49} Taken together, based on a manageable safety profile and promising survival data, lenvatinib will likely be approved for HCC in the foreseeable future and become an alternative in the first-line setting of patients with advanced HCC.

Cabozantinib, that targets several tyrosine kinases including MET, vascular endothelial growth factor (VEGF), and AXL, succeeded in a phase III (CELESTIAL) trial in sorafenib-experienced patients with advanced HCC and Child-Pugh class A.\textsuperscript{50} Up to two prior systemic therapies were allowed and sorafenib must have been one of them, meaning that cabozantinib was used as second or third-line treatment in this study. Cabozantinib significantly prolonged the primary endpoint median OS compared to placebo (10.2 vs 8.0 months) with a HR of 0.76 (95% CI, 0.63-0.92). The effect on PFS was even more pronounced (5.2 vs 1.9 months), as was the prolongation of median OS in patients who received sorafenib as only prior therapy (11.3 vs 7.2 months). The safety profile was acceptable with hand-foot-skin reaction and arterial hypertension being the most common grade ≥3 adverse events.\textsuperscript{50} Unlike in the phase III randomised controlled trial with regorafenib,\textsuperscript{46} this study also allowed the inclusion of patients intolerant to sorafenib.\textsuperscript{50} Table 1 summarises the survival data of all positive phase III trials of targeted therapies in HCC.

Taken together, lenvatinib was noninferior compared to sorafenib in terms of overall survival in the first-line setting and will be added to the treatment armamentarium shortly. No data are available on the efficacy of lenvatinib in patients with main portal vein invasion or a tumour load ≥50% of the liver as these patients were excluded from the study.

Regorafenib was the first drug to show a prolongation of survival in the second-line setting and has been approved for patients who have been previously treated with sorafenib. The magnitude of benefit was similar to that of sorafenib. Patients who progress on sorafenib are the best candidates for regorafenib while those intolerant to sorafenib may also not tolerate regorafenib very well. As the CELESTIAL trial allowed inclusion of patients who discontinued sorafenib due to adverse events, cabozantinib may become the preferred second-line option in patients intolerant to sorafenib, once approved by regulatory agencies.

Notably, as none of these agents was tested in Child-Pugh B patients, only patients with well-preserved liver function may be treated until data for Child-Pugh B patients become available and support the use of these drugs in this indication. The limited efficacy of the different agents (Table 1) may partly be owed to the lack of a biomarker for treatment selection, as discussed in the next paragraph.

### 2.4 Biomarkers for patient selection

Unlike “all-comer” trials, study inclusion based on oncogenic drivers or biomarkers for activated signalling pathways ensures that the molecular target is present in the studied population,\textsuperscript{24} which eventually may increase the likelihood of a positive outcome. Biomarker-driven treatment concepts have already been established for several malignancies (eg breast cancer, colorectal cancer, lung cancer) and have helped to improve the outcome of patients receiving systemic therapy.\textsuperscript{51-54}

Potential biomarkers in HCC include well-established prognostic markers like AFP, markers of key signalling pathways, or epigenetic markers among others. The RAS, mammalian target of rapamycin (mTOR), MET, and fibroblast growth factor (FGF)-19 signalling represent some of the few potential drivers of HCC progression, for whom selective inhibitors are already available for clinical use.\textsuperscript{24}

The mTOR inhibitor everolimus was tested in a phase III trial in the second-line setting against placebo.\textsuperscript{42} This pathway is activated in around half of all HCCs and associated with a worse outcome.\textsuperscript{55,56} Given the high frequency of aberrant mTOR signalling in HCC, one could expect at least some improvement in OS if everolimus was active, even though the study was performed in “all-comers”.\textsuperscript{42} Despite this strong theoretical rationale for the use of everolimus in HCC, final results did not even show a trend in survival (everolimus vs placebo, 7.6 vs 7.3 months).\textsuperscript{42}

A proof-of-principle trial evaluated the MEK inhibitor refametinib alone or in combination with sorafenib in patients with RAS-mutated HCC.\textsuperscript{57} The study was based on promising results of a phase II study showing better response rates for the combination of refametinib plus sorafenib in HCC patients with mutated RAS compared to wild-type RAS.\textsuperscript{58} Of 498 patients in the monotherapy and 820 patients in the combination arm included, the prevalence of RAS mutation was 6.5% and 3.3%, respectively, and 16 patients in each group finally received treatment. Given the insufficient efficacy with no confirmed response for refametinib alone and only one confirmed partial response for the combination arm this approach was not further pursued in HCC.\textsuperscript{57}

The MET inhibitor tivantinib was investigated in a second-line randomised controlled phase II study where it only improved survival...
in patients with high tumoral MET expression but not in cases with MET-low HCC.59 These results prompted the conduction of a phase III trial (METIV-HCC) testing tivantinib vs placebo only in patients with high MET expression.60 However, the high expectations for tivantinib to become the first biomarker-driven treatment approach in HCC could not be fulfilled, as recently presented results did not show an improvement of the primary endpoint OS (median OS for tivantinib vs placebo, 8.4 vs 9.1 months).60 Tivantinib also failed in a similar phase III (JET-HCC) study conducted in Japan only.61

While brivanib, a multitryosine kinase inhibitor with activity against FGF receptors, failed in two phase III trials in unselected “all-comers”,39,41 pilot studies using biomarkers for activated FGF signalling are under way. The FGF receptor blocker erdafitinib is currently being evaluated in an early phase study of advanced HCC patients with FGF19 amplification (NCT02421185). Another phase II/I study is testing the FGF receptor-4 inhibitor FGFO401 alone or in combination with an antiprogrammed cell death 1 (PD-1) antibody in sorafenib-experienced HCC patients with positive FGF receptor-4 and klotho beta (= co-factor for FGF19 activation) expression (NCT02325739).

Ramucirumab, a monoclonal antibody against VEGF receptor-2, was investigated in a second-line phase III randomised controlled trial (REACH). While ramucirumab failed to improve the primary endpoint OS in the whole cohort, patients with elevated serum AF had much better on ramucirumab than on placebo (median OS, 7.8 vs 4.2 months).43 Based on these data, another phase III randomised controlled trial (REACH-2) has been conducted testing ramucirumab vs placebo in sorafenib-experienced patients with AFP of 400 ng/mL or higher. As recently reported, ramucirumab moderately but significantly prolonged survival (median OS, 8.5 vs 7.3 months) with a HR of 0.71 (95% CI, 0.53-0.95), making ramucirumab the first drug that showed a survival benefit in a biomarker-selected population.62 Hence, ramucirumab—the first biomarker-guided treatment in HCC—will likely become a preferred option in patients with AFP ≥400 ng/mL, and especially in those with poor tolerance to tyrosine kinase inhibitors.

The difficult mission of setting up successful biomarker-enriched trials in HCC is at least in parts a result of the complex tumour biology. HCC is heterogeneous and the molecular profile obtained by a single biopsy does not guarantee that it is actually representative for the whole tumour load, especially in multifocal HCC.22 Additionally, the expression of a target or the activation of a certain signalling cascade does not categorically imply tumour dependency.64 These aspects further complicate the identification of tissue biomarkers to guide treatment decisions in HCC. Nevertheless, all efforts towards a personalised HCC therapy should be made and acquiring tissue samples for molecular profiling seems to be indispensable to achieve this goal.65

3 | IMMUNOTHERAPY

3.1 | Rationale

Immunotherapy has become a mainstay in the treatment of certain malignancies including melanoma and lung cancer.66 There are also several reasons why immunotherapy may be feasible and effective in HCC.67,68 HCC is an immunogenic tumour as spontaneous regressions, often immune mediated,68,69 and naturally occurring tumour-associated antigen-specific CD8+ T-cell responses, correlating with survival,70 have been reported. Several mechanisms in the tumour microenvironment create an immunosuppressive milieu (eg cytokines with suppressor function, immune checkpoints, defective antigen presentation, immunosuppressive cell types) which promotes tumour immune evasion,67,68,71,72 an emerging hallmark of cancer.73 The tolerogenic liver environment, essential to avoid overreaction to antigens delivered from the intestine,74 may further facilitate tumour immune escape.67,68,71 Chronic inflammation, present in most HCC cases due to an underlying liver disease/cirrhosis,3 also promotes T-cell exhaustion (hyporesponsive cells with impaired cytotoxicity) and immunosuppression.71 Finally, immunotherapeutic drugs are not metabolised by the liver making their pharmacological profile more predictable in patients with liver cirrhosis,67 a condition often found in patients with HCC.3

Several different approaches of immunotherapy exist, including vaccines, cytokines, oncolytic viruses, adoptive cell therapy, gene therapy, and immune checkpoint inhibitors.75 The latter ones will be discussed herein, as this strategy is already in advanced clinical testing in HCC and clinical data from early trials have been promising.

3.2 | Immune checkpoint blockers

Immune checkpoint receptors (eg PD-1, cytotoxic T-lymphocyte-associated protein 4 (CTLA-4)) and their ligands (eg programmed cell death 1 ligand 1 or 2) are membrane-bound coinhibitory molecules that are expressed on several cell types of the innate and acquired immune system, including T cells, natural killer cells, and dendritic cells. Physiologically, binding of a ligand to its receptor on the surface of T cells inhibits T-cell overactivation during an immune response in order to minimise collateral tissue damage.72,76,77

One important mechanism how tumours manage to escape host immunity is the expression of immune checkpoint molecules on the surface of cancer cells and cells of the tumour microenvironment (eg tumour-associated macrophages, regulatory T cells, myeloid-derived suppressor cells).67 Immune checkpoint blockers are monoclonal antibodies that interfere with the ligand-receptor interaction and thereby promote activation of immune effector cells in order to fight cancer cells.72

Several checkpoint inhibitors have already been tested in HCC (Table 2). Tremelimumab, an antibody against CTLA-4, was tested in a small pilot trial of 21 sorafenib-naive or -experienced patients (24%) with HCC and chronic hepatitis C virus infection.75 Of 17 patients evaluable, three had partial response (PR) and the disease control rate (DCR) was 76%. While median TTP was 6.48 months, the median OS of 8.3 months was less promising, but may be partially explained by a high number of Child-Pugh B patients (43%) included. The safety profile was mild with rash, fatigue, elevated transaminases, and diarrhoea being the most frequent side effects; only a few treatment-related AEs grade 3 or higher were reported.78
TABLE 2 Results of selected studies testing immune checkpoint inhibitors in hepatocellular carcinoma

| Author, year | Treatment (no. of patients) | Prior sorafenib treatment (%) | ORR/DCR (%) | TTP/PFS (months) | OS (months) |
|--------------|-----------------------------|-----------------------------|-------------|----------------|------------|
| Sangro 201378 | Tremelimumab (21)           | 23.8                        | 17.6/76.4   | 6.48/NR        | 8.2        |
| Duffy 201779  | Tremelimumab + subtotal ablation (32) | 65.6                        | 26.3/NR     | 7.4/NR         | 12.3       |
| Crocenzi & Sangro 201781,82 | Nivolumab (80) | 0                           | 22.5/62.5   | NR/NR         | 28.6       |
| Crocenzi & Sangro 201781,82 | Nivolumab (182) | 100                         | 18.7/62.6   | NR/NR         | 15.6       |
| Wainberg 201779 | Durvalumab (40)          | 92.5                        | 10/32.5     | NR/2.7         | 13.2       |
| Kelley 201785  | Durvalumab + Tremelimumab (40) | 75.0                        | 25/57.5     | NR/NR         | NR         |
| Zhu 201883    | Pembrolizumab (104)        | 100                         | 17.3/61.5   | NR/4.9         | 12.9       |
| Ikeda 201881  | Pembrolizumab + lenvatinib | 13.3                        | 42.3/100    | NR/9.7         | NR         |
| Stein 201892  | Atezolizumab + bevacizumab (43) | 0                           | 65/96%      | NR/NR         | NR         |

DCR, disease control rate; NR, not reported; ORR, overall response rate; OS, overall survival; PFS, progression-free survival; TTP, time to progression.

Preliminary results of a phase I/II trial investigating durvalumab, a mAb against programmed cell death 1 ligand 1 (PD-L1), in 40 patients (92.5% received prior sorafenib) with advanced HCC were recently reported.79 Four patients had PR (all confirmed), which occurred early and were durable; the DCR at ≥24 weeks was 32.5%. Median PFS and OS were 2.7 months and 13.2 months, respectively. Most common AEs were fatigue, pruritus, and elevated aminotransferases. Eight patients developed treatment-related AEs grade 3 or 4.79 Based on these data, a decision to develop durvalumab in combination with the CTLA-4 inhibitor tremelimumab as first-line treatment in advanced stage HCC was taken (see below).

The Checkmate 040 study is a phase I/II (dose escalation/dose expansion) trial that investigated nivolumab, a mAb against PD-1, in sorafenib-naive (n = 80) and sorafenib-experienced (n = 182) patients with intermediate-advanced HCC and Child-Pugh stage A. The primary endpoints were safety and investigator-assessed overall response rate (ORR) according to RECISTv1.1.80 Nivolumab was well tolerated with 29% (sorafenib-naive) and 18% (sorafenib-experienced) of patients experiencing grade 3/4 AEs. Most common side effects were fatigue, pruritus, rash, and diarrhoea. ORR was 22.5% in sorafenib-naive and 18.7% in sorafenib-experienced patients; most notably and different from previous trials with tyrosine kinase inhibitors, seven (2.7%) subjects had complete response (CR). Responses were meaningful and independent of PD-L1 expression and baseline serum AFP levels. Median duration of response was 17.0 months in the dose escalation phase and 9.9 months in the dose expansion cohort. The DCR was around 63%.80-82 These promising antitumour responses translated into encouraging survival results with a median OS of 28.6 months in sorafenib-naive and about 15 months in sorafenib-experienced patients.81 Based on these promising data, the United States Food and Drug Administration granted accelerated conditional approval to nivolumab for HCC patients pretreated with sorafenib; conditional indicates the final approval will depend on the results of an ongoing phase III trial of nivolumab vs sorafenib in the first-line in advanced HCC (NCT02576509).

The KEYNOTE-224 is a single-arm study testing pembrolizumab, another PD-1 antibody, in patients with intermediate-advanced HCC and Child-Pugh stage A who were previously treated with sorafenib.83 Of 104 patients reported to date, 1 patient had CR and 17 subjects had PR (ORR, 17.3%). Median PFS and OS were 4.9 months and 12.9 months respectively. Most common side effects included pruritus, fatigue, diarrhoea, and rash. Twenty-six percent of patients developed AEs ≥3.83

Taken together, results from uncontrolled phase I/II studies testing checkpoint inhibitors in HCC are encouraging but need to be confirmed in large randomised controlled trials. Table 3 provides a list of phase III studies evaluating checkpoint inhibitors in HCC.

3.3 Combination strategies with immune checkpoint inhibitors

Several strategies combining immune checkpoint blockers with other treatment modalities are under investigation for different stages of HCC, and the combination of different checkpoint blockers in advanced stage disease is one of them.

The combined use of nivolumab and ipilimumab (anti-CTLA-4) improves not only the efficacy in melanoma patients compared to ipilimumab alone but also increases toxicity.84 The combination of these compounds is currently under investigation in HCC (NCT01658878).

Durvalumab combined with tremelimumab is being tested in a large four-arm phase III study as first-line treatment in patients with unresectable HCC (NCT03298451) (Table 3). Preliminary results of a phase I/II study combining durvalumab and tremelimumab in advanced HCC were recently reported. Of 40 patients (75% received prior sorafenib) evaluable, 10 had PR (7 with confirmed PR); the DCR at ≥16 weeks was 57.5%. Most common side effects were manageable and included pruritus, diarrhoea, elevated transaminases, and rash. Ten patients (25%) experienced treatment-related AEs ≥3 or serious AEs.85

All tyrosine kinase inhibitors with proven efficacy in HCC20,46,49,50 target VEGF signalling among other pathways. VEGF can exert immunosuppressive effects on the one hand but anti-VEGF therapy can induce tumour hypoxia on the other hand.86 Hypoxia supports immunosuppression, inter alia by an upregulation of immune checkpoint molecules, and promotes tumour growth and
dissemination. Indeed, sorafenib induced tumour hypoxia and up-regulated PD-L1 expression in an experimental model of HCC. Triple combination of sorafenib, AMD3100 (C-X-C chemokine receptor type 4 inhibitor), and anti-PD-1 increased infiltration of cytotoxic T lymphocytes and delayed tumour growth and metastasis. Thus, combining targeted therapies with checkpoint blockers may represent a reasonable strategy and is currently tested in several clinical trials (eg PDR001 and sorafenib (NCT02988440), pembrolizumab and lenvatinib (NCT03006926), pembrolizumab and regorafenib (NCT03347292)). Recently presented preliminary data of a phase Ib study testing pembrolizumab plus lenvatinib in unresectable HCC showed a good safety profile, an encouraging ORR of 42.3%, and a median PFS of 9.69 months (Table 2). Similarly, the combination of atezolizumab (anti-PD-L1) and bevacizumab was well tolerated and showed promising preliminary efficacy results (ORR, 65%) as a first-line treatment in advanced HCC (Table 2).

Combination of checkpoint blockers with treatment modalities that increase the release of neoantigens (eg radiotherapy, loco-regional treatment) may further increase the efficacy of immunotherapy. A pilot study investigated the combination of subtotal ablation (local ablation or TACE) and tremelimumab in 32 patients (21 received prior sorafenib) with intermediate-advanced stage HCC, based on the hypothesis that tremelimumab may enhance an peripheral immune response induced by the ablative procedure. Of 19 evaluable patients who had target lesions that were not ablated, five achieved a confirmed PR lasting between 7 and 19 months. Median TTP and OS were 7.4 months and 12.3 months, respectively. Notably, tumour biopsies at week 6 demonstrated increased infiltration of intratumoral CD8+ T cells in responders. To what extent the ablative procedure contributed to the observed clinical effects needs further investigation. The combination of TACE (NCT03143270) or radioembolisation (NCT03033446, NCT02837029) with checkpoint inhibitors is currently studied in pilot studies.

Efficacy of immunotherapy is impaired by a major barrier—the immunosuppressive microenvironment. Renin-angiotensin system inhibitors—partly due to their antifibrotic/antidesmoplastic effects—may have the potential to reprogramme the immunosuppressive tumour microenvironment towards a more immunostimulatory milieu. This could enhance the efficacy of immunotherapy. Indeed, experimental models demonstrated that antifibrotic drugs improved the efficacy of anti-PD-1-targeted immunotherapy in HCC and other tumours. This strategy needs prospective evaluation in clinical trials. The transforming growth factor (TGF)-β inhibitor galunisertib, that also has antifibrotic potency, is currently being tested in combination with nivolumab in HCC (NCT02423343). Notably, TGF-β also promotes immunosuppression by inhibiting T-cell responses, which makes this combination particularly attractive.

### 4 CONCLUSIONS AND FUTURE PERSPECTIVES

As of today, two tyrosine kinase inhibitors have been approved for the treatment of HCC, namely sorafenib in the first-line and regorafenib in the second-line setting. Additionally, the immune checkpoint inhibitor nivolumab was recently conditionally approved for sorafenib-experienced HCC patients in the United States. With lenvatinib in the first-line and cabozantinib and ramucirumab in sorafenib-experienced patients, two more tyrosine kinase inhibitors and one monoclonal VEGF receptor-2 antibody have reached their primary endpoint in phase III randomised controlled trials and may soon be added to the armamentarium of systemic therapies for HCC. Which drug to choose first in each setting will depend on biomarkers like AFP for ramucirumab as the first biomarker-driven approach in HCC, but otherwise will be left to clinicians, further studies, and most importantly real-world data on their true tolerability. Upcoming results from phase III trials will show if immunotherapy will become a mainstay in the treatment of HCC. Studies investigating immunotherapy as (neo)adjuvant treatment in the curative setting (eg before or after resection or ablation) and approaches combining immunotherapy with other treatment modalities (eg tyrosine kinase inhibitors, loco-regional therapies) may reveal further potential of immunotherapy in HCC. Whether immunotherapy may be an option in patients with HCC recurrence after liver transplantation is also subject to further studies. Notably, a patient with pulmonary recurrence after living donor liver transplantation for HCC, who progressed on sorafenib showed complete radiological remission to pembrolizumab despite ongoing immunosuppression and without signs of graft rejection. However, nivolumab led to fatal
acute liver organ rejection in two patients with recurrent, metastatic fibrolamellar HCC.\textsuperscript{100}

Some biomarkers (eg AFP, VEGF, hepatocyte growth factor) may have prognostic implications,\textsuperscript{101} but only serum AFP was so far successful as biomarker to guide treatment decisions in HCC.\textsuperscript{62}

Biomarkers to monitor treatment efficacy are lacking. Decreases in serum AFP or VEGF under sorafenib treatment were associated with better response and survival in small cohorts,\textsuperscript{102-105} but large prospective studies for validation are lacking.

MicroRNAs and exosomes—which often contain microRNAs themselves—have been investigated in HCC in recent years, but mainly as diagnostic biomarkers.\textsuperscript{106,107} Some microRNAs may enhance and others decrease sorafenib sensitivity of HCC cells,\textsuperscript{108} and the expression of certain microRNAs in tissue\textsuperscript{109,110} and serum\textsuperscript{111,112} samples correlated with response to sorafenib therapy and survival. Again, these potential biomarkers have not been adopted into routine clinical practice yet, as data were often obtained from small retrospective studies and thus need further prospective validation.

A renaissance of the tumour biopsy may be inevitable to achieve the unmet need of individualised therapy,\textsuperscript{65} and acquisition of tumour tissue and serum samples for biomarker analysis should become a routine in clinical HCC trials.

Liquid biopsy could become an alternative to tissue biomarkers as it may provide an even more comprehensive profile of the cancer than that derived from small tumour specimens.\textsuperscript{113} However, unlike in other solid tumours such as lung cancer,\textsuperscript{114} liquid biopsy is not yet ready for clinical use in HCC, since large-scale studies using standardised techniques and uniform methodology are lacking.\textsuperscript{115} It also remains unclear if liquid biopsy can adequately reflect the immune tumour microenvironment.

Biomarkers to predict response to checkpoint blockers are also lacking in HCC. Neither tumoral PD-L1 expression nor baseline AFP predicted response to nivolumab in HCC.\textsuperscript{80,81} The intratumoral infiltration of CD8+ T cells during tremelimumab treatment was associated with better outcome in a pilot study; however, only a few patients were evaluable and serial tumour biopsy is required,\textsuperscript{93} thus, limiting its use in clinical routine. Other approaches to predict response to immunotherapy including gut microbiota\textsuperscript{116,117} or monocytes in the peripheral blood\textsuperscript{118} need further prospective evaluation.

**Acknowledgements**

Declaration of personal interests: M.P. is advisory board member of Bayer, BMS, Ipsen, and Eisai, and received travel support from Bayer, and speaking fees from Bayer and BMS. He is also an investigator for Bayer, BMS, and Lilly. M.P.R. received grant support and honoria from Bayer HealthCare and BMS, and served as a consultant for Bayer HealthCare, BMS, Lilly, ONXEO, Eisai, and Ipsen.

Declaration of funding interests: None.

**Authorship**

Guarantor of the article: Matthias Pinter.

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