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REVIEW

# Ramucirumab as a second line therapy for advanced HCC: a significant achievement or a wasted opportunity for personalised therapy?

Giandomenico Roviello[1] · Navid Sohbani[2] · Roberto Petrioli[3] · Maria Grazia Rodriquenz[4]

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**Summary**
The second line treatment of hepatocellular carcinoma (HCC) has recently become an exciting area of interest since new emerging options have demonstrated survival benefits versus placebo. Unfortunately, predictive biomarkers are unavailable for these treatments. Ramucirumab, a monoclonal antibody against VEGFR-2, has demonstrated overall survival superiority against placebo as a second line therapy for patients with AFP > 400 ng/ml in the recent REACH-2 trial. This review will provide the current updated knowledge regarding the HCC cancerogenesis and angiogenic VEGF/VEGFR-2 pathways and the clinical development of ramucirumab in advanced HCC. This study will also critically assess the gaps in a previous negative phase III trial that tested other potentially useful treatments and suggest ways to modernise clinical trials and personalise therapy for advanced HCC.

**Keywords** Ramucirumab · HCC · Alphafetoprotein

## Introduction

Hepatocellular carcinoma (HCC) is the fifth most common malignancy worldwide and the second leading cause of death from cancer (http://globocan.iarc.fr/old/FactSheets/cancers/liver-new.asp) [1]. Despite the implementation of surveillance programs and HBV vaccination, HCC incidence has been growing globally. In the last 20 years, newly diagnosed HCC cases increased by 75%; aging, greater life expectancy, and population growth are the main causes of the recent rise in the incidence of HCC [2, 3]. Most patients are diagnosed at an advanced stage, which is characterised by limited therapeutic options and poor median survival.

Several new agents have been investigated in the last decade after the approval of sorafenib. Despite promising data in early phases of drug development, both in preclinical studies and phase I and phase II clinical trials, most of these medications failed to show significant clinical benefits in larger phase III randomised studies in both first and second line in unselected populations, for either non-inferiority (brivanib and linifanib [4, 5]) or superiority trial design (sunitinib and erlotinib plus sorafenib in first line and brivanib, everolimus, and tivantinib in second line [6–10]).

The treatment landscape for patients with HCC has changed since the FDA and EMA approved the multikinase inhibitor regorafenib in second line. Cabozantinib, also a multikinase inhibitor, will join regorafenib as an option in the second line setting according to positive results of the phase III CELESTIAL trial presented in January 2018 at ASCO GI [11]. Very recently, in a first line setting, lenvatinib, another antiangiogenic multikinase inhibitor, demonstrated non-inferiority in overall survival (OS), progression-free survival (PFS), and objective response rate (ORR) compared to sorafenib in the phase III REFLECT trial [12].

Moreover, high anticipation has accompanied more recent results on the use of immune checkpoint inhibitors in

✉ Giandomenico Roviello
giandomenicoroviello@hotmail.it

[1] Department of Health Sciences, University of Florence, viale Pieraccini, 6, 50139 Florence, Italy

[2] Department of Medical, Surgery and Health Sciences, University of Trieste, Piazza Ospitale 1, 34129 Trieste, Italy

[3] Medical Oncology Unit, Department of Medicine, Surgery and Neurosciences, University of Siena, Siena, Italy

[4] Division of Medical Oncology, Department of Onco-Hematology, IRCCS-CROB, Referral Cancer Center of Basilicata, via Padre Pio 1, 85028 Rionero, Vulture, PZ, Italy

advanced HCC. Based on a single-arm phase II trial, in September 2017, the FDA granted accelerated approval for nivolumab for HCC previously treated with sorafenib [13]. The ORR (15–20%) and median OS reported (16 months) for nivolumab after sorafenib are significantly higher than those with TKIs and have heightened expectations for significant improvement in advanced HCC treatment after TKIs. These new advances emphasise the importance of identifying the alterations at a molecular level that drive HCC cancerogenesis and progression to attain an optimised personalised approach to HCC.

Despite the rising number of molecular targeted therapies now available for HCC, to date no biomarkers have been discovered. Due to the molecular heterogeneity of HCC, one of the main challenges of HCC is the identification of crucial oncogenic pathways that would facilitate patient selection for targeted therapy.

The results from the phase III REACH-2 trial demonstrated a significant survival benefit of second line ramucirumab treatment in patients with HCC and alpha-fetoprotein (AFP) ≥ 400 ng/ml who had previously received sorafenib [14]. This represents the first positive trial in a biomarker-selected subgroup and is expected to usher in a new era of HCC personalised approach.

## HCC CORE hallmarks in the "MULTIOMICS" era

HCC carcinogenesis is a long and multistep process triggered by multiple risk factors that contribute to HCC heterogeneity, including viral hepatitis, chronic alcohol abuse, non-alcoholic fatty liver, and other metabolic diseases such as diabetes [15]. Regardless of the HCC aetiology, nearly all HCC cancerogenesis starts with a background chronic inflammation or cirrhosis that can cause chronic cell damage and consequently results in the accumulation of genomic alterations and the activation of several signalling pathways. Together with the chronic inflammation of the hepatic microenvironment, the induction of angiogenesis is one of the most remarkable hallmarks of HCC and constitutes the main target of available therapies. Furthermore, the tumour microenvironment (TME) and the crosstalk with tumour cells are thought to play roles in the pathogenesis of HCC [15].

Next-generation genomic technologies have certainly elucidated the molecular characterisation of core oncogenic pathways within the tumour cell population, contributing to the development of a biological and prognostic classification of HCC. However, genome sequencing analyses have focused less on the corresponding TME that is actually the main target of antitumoural therapies. Several studies on deep genome sequencing of approximatively 1000 HCC samples have demonstrated approximately 30–40 mutations per tumour, although 5–8 might be driver mutations [16].

The key mutations in HCC occur in genes that are primarily involved in four oncogenic networks: metabolic processes, WNT-β-catenin signalling, chromatin modification, and PI3K-AKT-mTOR activation [17]. More than half of HCCs harbour mutations in telomerase reverse transcriptase (TERT) promoters (56%), particularly those involved in the control of cell-cycle and senescence. Other common mutations occur in the TP53 (27%), CTNNB1 (26%), ARID2 (7%), ARID1A (6%), and AXIN1 (5%) genes. The CTNNB1 and AXIN1 genes are members of the WNT pathways that are specifically involved in cell proliferation, stem cell maintenance, and other oncogenic signals, and CTNNB1 in particular is involved in the activation of WNT signalling [16]. Unfortunately, the majority of pathways remain "undruggable."

Amplifications of oncogenes in chromosome regions 11q13 (CDND1 and FGF19) and 6p21 (VEGF-A) have been identified in 5–10% of HCCs as well as amplifications in MYC proto-oncogene (MYC), mesenchymal-epithelial transition factor (MET), and focal amplifications in TERT promoter genes [18, 19].

Several links can be found between intracellular pathways and neoangiogenic angiogenic processes. For instance, the PI3K/AKT/mTOR pathway activates various tyrosine kinase receptors, such as VEGFR, EGFR, and PDGFR. In addition, RAS/RAF/MEK/ERK signalling, constitutively activated in HCC, modulates crucial cellular processes, such as proliferation, differentiation, cell adhesion, and angiogenesis [20].

More than 40 prognostic and predictive HCC gene signatures have been identified over the last decade, predominantly in surgically resected HCC tissues, and the list continues to grow, although none have been validated for clinical decision-making [21, 22]. Genetic studies are mainly employed to discover useful biomarkers for risk stratification in early diagnosis, recurrence, and prognosis and to identify pathways involved in cancerogenesis and invasion/metastases that may lead to targeted therapy based on the patient's own genetic profile.

A primary attempt at molecular HCC classification identified two major molecular subclasses, proliferative and non-proliferative genotypes, regardless of aetiology and ethnicity, each representing approximately 50% of HCCs [23, 24]. The proliferative subclass is characterised by the inactivation of TP53 and the activation of the RAS, mTOR, and IGF pathways and correlates with poor prognosis and AFP overexpression. The non-proliferative subclass has less aggressive disease progression and is heterogeneous both in epidemiological and molecular profiles.

In 2009, Hoshida et al. published the first consensus classification framework for HCC based on the collection of all 8 pre-existing HCC datasets plus a new dataset, totalling 603 patients [24]. Three HCC subclasses were observed (called S1, S2, and S3), each with peculiar clinical features, such as tumour size and serum AFP levels. At the molecular level, the

S1 subclass is characterised by WNT signalling pathway activation through TGF-β. The S2 subtype showed MYC and AKT activation, overexpression of IGF2, and induction of the EpCAM signature and is associated with elevated AFP. The pattern of protein expression in the S2 subtype, which includes FGFR3, FGFR4, and IGF2, might enhance VEGF/VEGFR-2 pathway activity and influence sensitivity to ramucirumab. Both the S1 and S2 subtypes correlated with poor prognosis and are included in the "proliferative" phenotype. The S3 subclass showed activated mutations in the CTNNB1 gene and was associated with hepatocyte-like phenotype, superior differentiation, a better prognosis, and low proliferation.

In addition to those derived from tumours, a number of studies have built signatures derived from adjacent cirrhotic tissue. These signatures likely catch oncogenic signals reflecting the so-called "field effect." Since mesenchymal signatures may be influenced by the sampling of non-representative regions, proper pathology quality control is required to guarantee the reproducibility of these kinds of gene expression signatures while avoiding "confounding noise". Genomic data from both components (that is, tumour and adjacent tissue) should be considered complementary to maximise prediction accuracy.

## ANGIOGENIC pathways in HCC

HCC is known as a hypervascular tumour as it is mainly supplied by the hepatic arteries, while the portal vein feeds normal liver parenchyma and regenerative and dysplastic nodules. This typical hypervascularity also regulates the pattern of distribution of HCC metastases, mainly growing in the primary tumour's drainage area via portal vein efferent flow [25] and is also used as a radiological characteristic in the diagnosis of HCC [26]. Thus, it could be asserted that angiogenesis plays a vital role in HCC initiation, growth, and metastatic dissemination [27] and therefore is a major target for therapy [28].

Angiogenesis is a highly complex and coordinated process mediated by a complex equilibrium between proangiogenic and antiangiogenic factors acting on both the tumour cells and endothelial cells. The best known angiogenic treatments for HCC are VEGF, PDGF, and FGF. All activate the RAS/RAF/MEK/ERK pathway and the PI3K pathway in the endothelial cells [29]. VEGF plays a central role in angiogenesis, and there is crosstalk among VEGF, FGF, and PDGF signalling that potentially may have therapeutic implications [30, 31].

VEGF promotes the activation and proliferation of endothelial cells that consequently impair the extracellular matrix. As a result, further angiogenic factors are released, such as angiopoietin (ANGPT)1, ANGPT2, fibroblast growth factor (FGF), and transforming growth factor-beta (TGF-β) [28]. These factors in turn promote the proliferation and migration of endothelial cells to form a new abnormal vascular network [32]. Through PDGFR activation, pericytes and smooth muscle cells can gather at the wall of the developing vessel contributing to neovascularisation. Moreover, FGF may contribute to HCC's angiogenesis by promoting the synthesis of matrix metalloproteinases (MMPs). On a microscopic level, HCC microvasculature typically appears arterialised, resembling capillaries, and is less dense than normal liver vasculature.

VEGF is not only released from tumour cells, but also from stroma cells and the extracellular matrix via matrix metalloproteinase (MMP)-9-mediated proteolysis [33]. Thus, VEGF may function as both an autocrine growth factor in cancer cells expressing VEGF receptors and a paracrine growth factor in the vasculature. VEGF can also act directly on hepatocytes, Kupffer cells, and hepatic stellate cells as a cytokine [34, 35]. Oncogenic genes, hormones, cytokines, hypoxia, and acidosis regulate VEGF expression [36, 37].

The VEGF pathway may also interfere with the immune system, causing systemic immunosuppression in cancer patients [38]. It has been reported that elevated levels of circulating VEGF might inhibit T cell immune responses by suppressing the maturation of dendritic cell precursors and promoting the proliferation of regulatory T cells [39].

VEGF exerts its biological effects via two receptor tyrosine kinases, VEGFR-1 (Flt1) and VEGFR-2 (Flk1/KDR) that show considerable differences in ligand affinity and activity. Although VEGFR-1 has an increased affinity for VEGF compared to VEGFR-2, the tyrosine kinase activity of VEGFR-2 is more effective by a factor of 10 and is the principal effector of mitogenic and angiogenic activity of VEGF [40]. After binding its ligand, VEGFR-2 undergoes dimerization and tyrosine phosphorylation and activates several intracellular signalling pathways, such as the RAS/RAF/MEK/MAPK, PI3K/AKT/mTOR, and JAK/STAT axes, thus exerting its role on vascular endothelial cells [40].

VEGF is the most studied angiogenic treatment for HCC. A number of studies described VEGF expression in HCC and a correlation between circulating and/or tissue VEGF and clinical outcomes. VEGF expression increases progressively from dysplastic nodules to early HCC supporting the hypothesis of VEGF-driven angiogenesis in hepatocarcinogenesis [41]. Furthermore, a significant correlation between the serum VEGF levels in patients with HCC and the tumour expression of VEGF (mRNA and protein expression) suggests the use of circulating VEGF as a prognostic marker [42].

In HCC, several studies of circulating VEGF and/or tissue overexpression have correlated with high HCC tumour grade, vascular invasion and portal vein invasion, rapid tumour recurrence, and poor prognosis [43, 44].

Data available on the prognostic role of VEGF expression were systematically examined in few meta-analyses. A meta-analysis published in 2009 by Schoenleber on 16 HCC studies of both high tissue and serum VEGF levels predicted poor

overall and disease-free survival [45]. A subsequent meta-analysis by Zhan et al. of 14 studies suggested that VEGF overexpression had a negative impact on OS but not disease-free survival (DFS), also due to significant heterogeneity among DFS [46] studies. Another meta-analysis of 9 studies specifically focused on the predictive role of VEGF in monitoring the efficacy of sorafenib. Some evidence showed that a high level of VEGF before sorafenib correlated with a good response, while a high level of VEGF after sorafenib administration correlated with a poor OS and a poor PFS in HCC [47].

A high level of VEGFR expression was observed in HCC cell lines and tissues as well as in the blood circulation of patients with HCC [48]. However, the pattern of VEGFR-2 expression in HCC tissue and the relationship between the level of VEGFR-2 expression and the clinicopathological characteristics in HCC remain unclear. A high expression of VEGFR-2 in HCC was related to poor prognostic clinical and pathological features such as a large tumour diameter, high serum AFP, poor differentiation, and 5-year survival [49]. Moreover, VEGFR-2 overexpression has been associated with the rapid progression of HCC [49].

Biomarker analysis from the SHARP trial evaluated the baseline concentrations of a set of 10 biomarkers, including VEGF, Ang-2, VEGF, sVEGFR-2, sVEGFR-3, HGF, s-c-KIT, insulin-like growth factor-2, all forms of circulating RAS, and their modifications over 12 weeks assessing the correlation with the prognosis or the response to treatment with sorafenib. Serum VEGF and Ang-2 levels were demonstrated to have independent prognostic values but were not predictive of the response to sorafenib. The median survival of patients with low and high baseline Ang-2 concentrations was 14.1 and 6.3 months, respectively. Serum VEGF levels were found to be better markers than tissue VEGF, although further studies are required to confirm this observation [50].

Despite confirming the crucial role of the VEGF pathway in hepatocarcinogenesis and tumour progression underlying its prognostic implications, no validated biomarker has been discovered that can be routinely utilised in clinics for HCC. This lack of biomarkers is certainly a major obstacle to the personalised management of patients affected by HCC.

## RAMUCIRUMAB in HCC

### Pharmacological features and BIOOLOGICAL rationale for cancer treatment

Ramucirumab is a fully human G1 monoclonal antibody that binds to human VEGFR-2 with an affinity eightfold superior to that of its natural ligand VEGF-A [51]. Ramucirumab binds both soluble and cell VEGFR-2, competing with VEGF to bind with the receptor. By blocking the interaction of VEGFR-2 and its ligand, ramucirumab can inhibit downstream signalling and, consequently, endothelial proliferation and migration [52]. Ramucirumab blocks all known VEGFs from binding to VEGFR-2, and this peculiarity differentiates ramucirumab from bevacizumab, which binds only to VEGF-A. This combination of high specificity for VEGFR-2 and high affinity for the target could drive major antiangiogenic activity [53, 54]. Thus, ramucirumab seems to have a biological advantage compared to tyrosine kinase inhibitors with their biochemical promiscuity, incomplete receptor blockade, and potential off-target toxicities (Fig. 1).

### Utility of RAMUCIRUMAB for cancer treatment

Ramucirumab demonstrated improving OS in advanced gastric and colon cancer, either alone or in combination with different chemotherapy regimens underlying that VEGFR-2 inhibition might represent a useful therapeutic target for several tumours. To date, ramucirumab is approved as a second line therapy for advanced gastric or gastroesophageal junction adenocarcinoma, either as a single agent or in combination with paclitaxel, in metastatic colorectal carcinoma in combination with a FOLFIRI regimen, and in metastatic non-small cell lung cancer in combination with docetaxel.

### The clinical development of RAMUCIRUMAB in HCC

Ramucirumab is the only antiangiogenic monoclonal antibody to have reached advanced phases of clinical trials in HCC. Preliminary evidence of potential anticancer activity by ramucirumab in this disease derived from tumour growth inhibition as shown in HCC animal models. In addition, two phase I studies tested ramucirumab to determine the maximum-tolerated dose (MTD) using doses ranging from 2 mg/kg/week to 20 mg/kg/3 weeks. Overall, 30% of patients with different primary tumours experienced disease control longer than 6 months, including two patients with advanced HCC who had disease control around and exceeding 1 year, respectively, with dose-limiting toxicities of hypertension and deep vein thrombosis [55, 56]. These results provided the rationale for the further clinical development of ramucirumab in HCC. Although it is substantially lower than the maximum tolerated dose (13 mg/kg/week), a dose of 8 mg/kg every 2 weeks was selected because of the efficacy observed in a phase I evaluation. From a biological standpoint, in preclinical models this dose was linked to minimum concentrations that exceeded levels associated with tumour growth inhibition and with pharmacokinetic profiles suggesting receptor saturation. Subsequently, ramucirumab was tested in a phase II trial as a first line monotherapy in 42 patients with advanced HCC showing a median PFS of 4.0 months, ORR of 9.5%, and median OS of 12.0 months. (The study started before the approval of

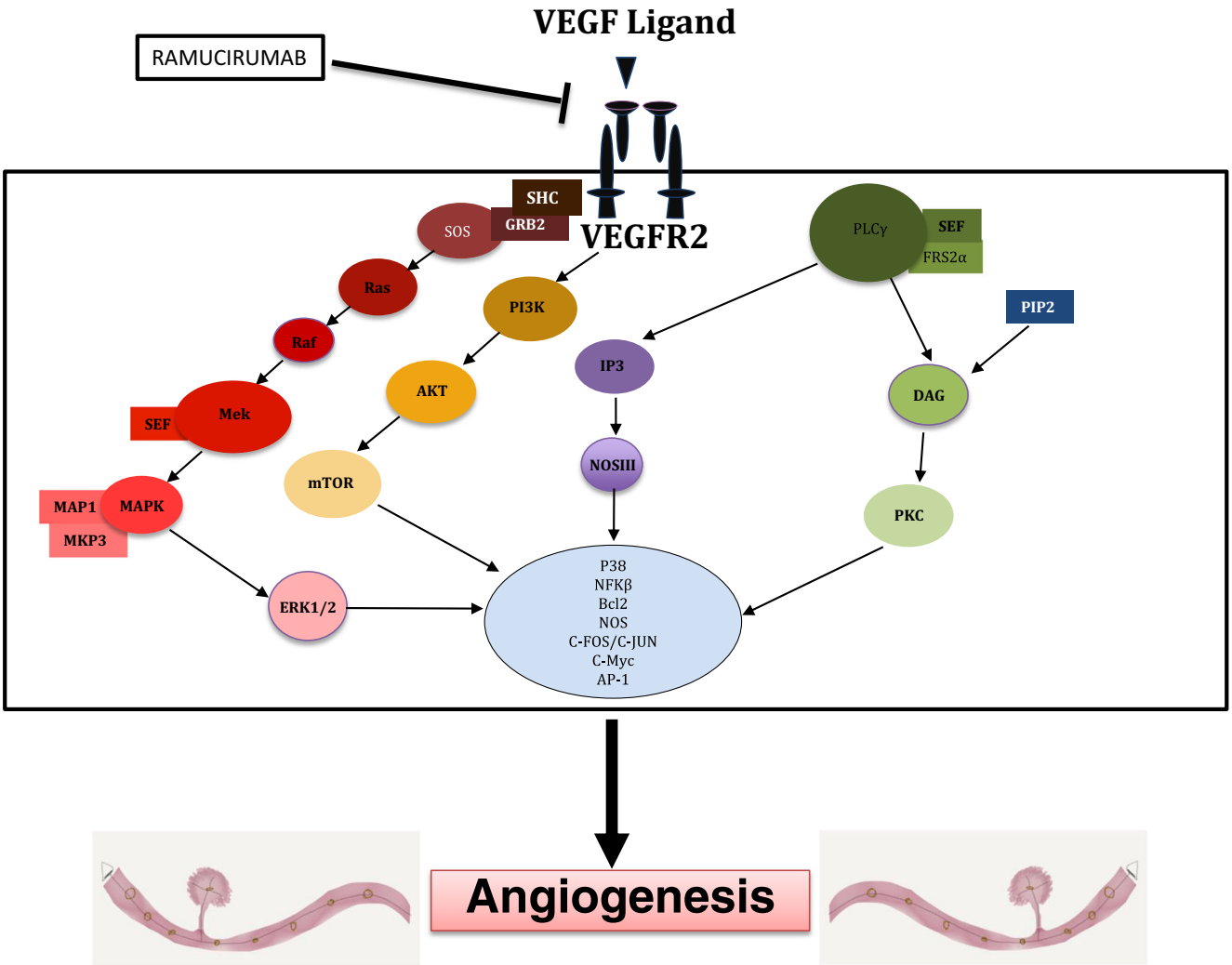


**Fig. 1** Ramucirumab and VEGFR-2 molecular pathways

sorafenib and was afterward considered appropriate after sorafenib approval due to the limited experience with sorafenib at the time) [56]. The most common grade 3 or more toxicities were hypertension (14%), gastrointestinal bleeding (7%), infusion-related reaction (7%), and fatigue (5%). Notably, patients who developed hypertension had better PFS and OS (PFS 4.2 vs 3.1 months; OS 23.1 vs 6.1 months). The investigators also performed parallel exploratory biomarker analyses that showed similar changes in circulating VEGF, PlGF, and sVEGFR-2 compared to other anti-VEGF-2 TKIs. An increase in the serum levels of VEGF and PlGF was observed, particularly after ramucirumab infusion. An exploratory analysis also showed a potential association between sVEGFR-1 modifications and both PFS and OS (with better outcomes in patients who experienced reductions in VEGFR-1 levels from baseline to day 8).

Despite the intrinsic limitations of the trial, such as the modest sample size and the single-arm design in a first line setting, it represented the first biomarker study of ramucirumab using data from a patient subset.

In the full extended paper, the investigators concluded that these exploratory data should have been considered hypothesis-generating although they must be confirmed in larger, prospective, randomised trials to clarify whether these biomarkers had prognostic and/or predictive values for ramucirumab use. Despite these translational assumptions, no further biomarker analysis was performed before the phase III trial.

## REACH trial

The first phase III trial of ramucirumab in HCC, known as the REACH trial, was conducted in patients with advanced HCC belonging to Child-Pugh class A HCC and Barcelona Clinic Liver Cancer (BCLC) stage B or C refractory but who were not candidates for locoregional therapy and who failed or were intolerant to first line sorafenib [57]. A total of 565 patients

**Table 1** Patient characteristics and results comparison among REACH, REACH2 trials and pooled analysis REACH2/REACH (AFP ≥ 400 ng/ml)

| | | REACH | | REACH2 | | Pooled analysis REACH2/REACH (AFP ≥ 400 ng/ml) | |
|---|---|---|---|---|---|---|---|
| | | Ramucirumab | Placebo | Ramucirumab | Placebo | Ramucirumab | Placebo |
| Age | Median | 64 | 62 | 64 | 64 | 64 | 62 |
| PS (ECOG) 0 | | 56% | 54% | 57% | 58% | 54.7% | 52.2% |
| Etiology | HBV | 35% | 36% | 36% | 38% | 39.2% | 45.1% |
| | HCV | 27% | 27% | 24% | 29% | 26.3% | 24.8% |
| Microvascular invasion | | 29% | 28% | 35.5% | 34.7% | 35.8% | 34.1% |
| Extraepatic spread | | 73% | 71% | 71.6% | 73.7% | 71.5% | 75.7% |
| Baseline AFP | median | 174 | 330 | 3920 | 2741 | 4104.6 | 4047.5 |
| OS | median (months) | 9.2 | 7.6 | 8.5 | 7.3 | 8.1 | 5 |
| | | HR 0.87 [95% CI 0.72–1.05]; $p = 0.14$ | | HR 0.71 [95% CI 0.531–0.949]; p = 0.0199 | | HR 0.694 [95% CI 0.571–0.842]; p = 0.0002 | |
| PFS | median (months) | 2.8 | 2.1 | 2.8 | 1.6 | 2.8 | 1.5 |
| | | HR 0.63 [95% CI 0.52–0.75]; p < 0.0001 | | HR 0.452 [95% CI 0.339–0.603]; $p < 0.0001$ | | HR 0.57 [95% CI 0.572–0.694]; $p = 0.0001$ | |
| ORR | | 7% | 1% | 5% | 1% | 5.4% | 0.9% |
| | | P < 0.0001 | | P = 0.1697 | | P = 0.004 | |

were enrolled in this trial and randomised to receive ramucirumab or placebo. Overall, 44% of patients had baseline AFP levels greater than 400 ng/mL (Table 1).

The REACH trial failed to demonstrate superiority in the primary endpoint, which was OS, in the experimental arm (ramucirumab vs placebo: 9.2 months vs 7.6 months; HR = 0.87 [95% CI = 0.72–1.05]; $p = 0{\cdot}14$) although the patients treated with ramucirumab had better ORR (7% vs 0.7%, $p < 0.0001$) and PFS (HR = 0.625, $p < 0.0001$) than those treated with placebo.

The REACH trial was a negative trial, but notably, in the preplanned subgroup with baseline serum AFP = 400 ng/mL, a survival benefit was observed in the treatment arm (7.8 months vs 4.2 months; HR = 0.674, $p = 0.006$) with a good safety profile. This trend was not observed in the patients with baseline AFP < 400 ng/mL, although a consistent improvement in PFS was shown in both the high and low baseline AFP groups. The patients with high serum AFP treated with ramucirumab experienced a delay in symptoms and performance-status deterioration together with an OS benefit. In addition, in the overall population, elevated AFP blood concentration was confirmed as a marker of poor prognosis. These findings suggested the existence of a potential subset of patients, driven by high serum AFP values, who might benefit anti-VEGF2 therapy.

## REACH-2 trial

To confirm the suggestion of a benefit of using ramucirumab for preselected populations with AFP > 400 ng/ml, the REACH-2 phase III trial was designed comparing ramucirumab vs placebo with a 2:1 ratio [14]. When the study began, no treatments were approved in the second line and therefore placebo was the competitor for ramucirumab. A total of Except for AFP levels (≥ 400 ng/ml required in REACH-2 trial), the eligibility criteria for both REACH-2 and REACH were similar and, as in the REACH trial, the primary endpoint was OS. The secondary endpoints were PFS, ORR, and safety. At a median follow-up of 7.6 months Ramucirumab significantly improved OS (median OS 8.5 vs 7.3 months; HR = 0.710; 95% CI = 0.531–0.949; $p = 0.0199$) and PFS (median PFS 2.8 vs 1.6 months; HR = 0.452; 95% CI = 0.339–0.603; $p < 0.0001$). The ORR did not differ significantly between the two groups (5% vs 1%; $p = 0.1697$), while the proportion of patients who experienced disease control rate was higher in the ramucirumab arm (59.9% vs 38.9%; $p = 0.0006$). The safety profile was in line with the well-known adverse events related to ramucirumab. The most frequent treatment-emergent adverse events of any grade in ramucirumab group were fatigue (27%), peripheral oedema (25%), hypertension (25%) and decreased appetite (23%), predominantly of grade 1–2. The only grade 3 or worse treatment-emergent adverse events in 5% or more of patients in ramucirumab group and significantly more frequent that in placebo group were hypertension and hyponatraemia. REACH-2 definitely met its primary endpoint showing a statistically significant survival benefit. Ramucirumab treatment reduced the risk of death by 29% in patients with AFP ≥ 400 ng/mL. REACH-2 is therefore considered the first positive phase III study conducted in a biomarker-selected patient population with HCC.

## Pooled analysis of the REACH and REACH-2 trials

An individual pooled efficacy and safety analysis using data across the two randomised phase III studies previously described (REACH-2 and REACH with baseline AFP ≥ 400 ng/ml) has been recently included in the full-length article of REACH-2 trial [14]. The eligibility criteria for both REACH-2 and REACH were similar, except for the AFP levels, proportion of patients with BCLC stage C disease and proportion of patients with HBV infection that were lower in REACH-2 than in REACH. Both of the studies enrolled patients with advanced HCC, Child-Pugh <7, ECOG PS ≤ 1, BCLC stage C or B refractory/not amenable to locoregional therapy, ≥ 1 measurable lesion, adequate hematologic and biochemical parameters, disease progression during or following, or intolerance to sorafenib. All of the patients received either ramucirumab (8 mg/kg IV) or placebo every 14 days until disease progression or unacceptable toxicity.

The pooled analyses included the assessment of OS, PFS, ORR, and safety. The efficacy analyses were stratified by study to account for any potential differences in the two studies.

A total of 542 patients were pooled respectively from REACH ($N = 250$) and REACH-2 ($N = 292$), with 316 patients receiving ramucirumab and 226 patients receiving placebo. Characteristics at baseline, including AFP, were well balanced between the arms (Table 1).

Consistently with the individual studies, the pooled analysis of efficacy showed that ramucirumab treatment significantly improved OS by a median 3.1 months (median OS 8.1 months vs 5.0 months; HR = 0.694; 95% CI = 0.571, 0.842; $p = 0.0002$). The analysis also showed improvements in PFS (median PFS 2.8 months vs 1.5 months with placebo; HR = 0.572; 95% CI: = 0.472, 0.694; $p < 0.0001$), ORR (5.4% vs 0.9% with placebo; $p = 0.0040$), and DCR (ORR + stable disease = 56.3% vs 37.2% with placebo; $p < 0.0001$).

Treatment discontinuation due to adverse events (AEs) related to study treatment occurred in 9.5% of the patients in the ramucirumab arm vs 3.6% in the placebo arm. The only grade ≥ 3 treatment-related adverse events occurring in ≥5% of the patients in the ramucirumab arm were hypertension (12.0% vs 3.6% with placebo) and hyponatraemia (5.1% vs 2.2% with placebo).

Regarding the subgroup analysis, ramucirumab's overall survival treatment benefit was consistent and robust across all of the subgroups and the sensitivity analyses including the random effect models were consistent. However, the benefits of ramucirumab were significantly better for men, for patients younger than 65 years, for white and Asian patients, for those with non-hepatitis B or C aetiology of liver disease, and for patients with extrahepatic metastases. In addition, patients without microvascular invasion, those with a BCLC score of C versus B, those with excellent baseline performance status, those who received prior locoregional therapy, and those who discontinued sorafenib because of disease progression also demonstrated benefits from ramucirumab [58].

## Emerging role of AFP as a predictive biomarker

Serum AFP is the biomarker most commonly used in HCC and is well known as both a diagnostic and prognostic marker [59]. The role of serum AFP testing in HCC surveillance settings and for diagnosis remains under investigation [3]. Elevated AFP levels constitute a risk factor for HCC development. Otherwise, AFP has a suboptimal performance as serological test for screening [60] and the addition of AFP dosages to US provides only a further detection rate of approximately 6–8% [61]. Two main reasons may explain the suboptimal role of AFP in surveillance. First, fluctuating levels of AFP might result not only from HCC development, but also from flares of HBV/HCV infection of the exacerbation of underlying liver disease [62]. Second, only a small percentage of early stage HCC (10–20%) has elevated AFP serum levels, a condition that has recently been correlated with an aggressive molecular subgroup of HCC (S2 class, EpCAM-positive) [63]. Conversely, the diagnostic accuracy of AFP significantly increased because of the reduction in false-positive cases at lower AFP thresholds (as low as 12–20 ng/ml) after removing viremic confounding factors due to pharmacological treatment.

Several clinical studies of advanced HCC have demonstrated that the serum AFP level was a potent prognostic factor, suggesting that AFP-positive HCC represents a subgroup with distinct biology. Elevated AFP is associated with larger tumours, poorly differentiated histology, bilobar involvement, portal vein invasion, and poor median survival. Serum AFP has also been integrated into some HCC prognostic scoring systems, such as CLIP, CUPI, and French staging systems [64].

Approximately half of all patients with advanced HCC have high levels of AFP (≥ 400 ng/mL) and this subgroup is known to have a poorer prognosis than the general HCC patient population. High AFP expression may be associated with increased angiogenesis through the elevated expression of VEGF and VEGFR-2, which potentially enhances tumour sensitivity to VEGFR-2 inhibition. Indeed, translational studies demonstrated a correlation between AFP expression and elevated VEGFR expression. Huang et al. found that the expression of VEGFR-2 in HCC cells was higher than in hepatic cells. Furthermore, the high expression of VEGFR-2 in HCC was related to a large tumour diameter, poor differentiation, and high serum α-fetoprotein [65].

In a cohort of HBV-related resected HCC cases, Yamashita et al. demonstrated that HCC can be stratified into four subtypes according to EpCAM and AFP status using different technical approaches (gene expression profiling, network pathway

analysis, and immunohistochemical analysis). These four subtypes underlie varying molecular subgroups of HCC with different prognoses and specific activated pathways. While the activation of Wnt-beta-catenin signalling was mainly associated with EpCAM + AFP + HCC, mature hepatocyte-specific genes are highly expressed in EpCAM – AFP + HCC. These findings suggested that these two types of aggressive HCCs are distinct and seem to share some molecular features with the S2 subclass; these different HCC subtypes should be managed with varying therapeutic interventions [66].

The biological mechanism that might explain the potential correlation between baseline AFP and the survival benefit of ramucirumab is uncertain. The molecular HCC S2 subclass is associated with elevated baseline AFP and poor survival. S2 is characterised by the expression of several kinases implicated in growth signalling, such as FGFR3, FGFR4, and IGF2 and its receptor, which might increase VEGF/VEGFR-2 pathway activity, thus influencing sensitivity to ramucirumab. Matching the correlation between elevated AFP with a high expression of VEGF and VEGFR-2 and the recent HCC genetic profile classification might suggest that high AFP expression is associated with a particular subtype of HCC with increased angiogenesis and possibly enhanced sensitivity to VEGFR-2 inhibition.

An elevated baseline AFP could simply be a better marker of poor prognosis in HCC that could benefit from ramucirumab or, according to a more sophisticated hypothesis, the potential association between ramucirumab efficacy and elevated baseline AFP could be part of the unique selective inhibition of VEGFR-2 by ramucirumab.

In the REACH trial, both the high and low baseline AFP groups treated with ramucirumab demonstrated a significant improvement in PFS, but only the patients with high baseline AFP experienced better overall survival. To explain this discrepancy, it may be assumed that in patients with high baseline AFP, HCC is more likely the cause of death. Conversely, in patients with low baseline AFP, other factors could be a more frequent cause of death, such as underlying liver disease and cirrhosis, which might explain the absence of a survival benefit despite the increase in progression-free survival and disease control with ramucirumab.

## Personalised therapy in HCC: The lessons learned from previous failed clinical trials

### Reasons for previous study failures of antiangiogenic drugs

The recent results of the REACH-2 trial and the identification of AFP as predictive biomarker have renewed the interest in patient selection criteria and critical analyses regarding other treatments, predominantly TKIs, which although have strong biological rationales have failed phase III trials in HCC. In fact, several agents showed antitumour activity in phase II trials that did not translate into OS improvement in following phase III trials, both in first line (brivanib, sunitinib, erlotinib, and linifanib) and second line (brivanib and everolimus) settings. These studies did not enrol patients based on the predictive markers of response, ignoring the complex heterogeneity of this disease.

A number of potential reasons should explain these trials' failures, indicating that some of the treatments could be active and effective only if used in the appropriate context. The reasons for trial failures in HCC may be reassumed and classified into three main categories: tumour-related factors, liver-related factors, and improper study design.

1- *Tumour-related factors*

– *Complex intertumour heterogeneity of HCC*

The prognosis of advanced HCC is influenced by a number of factors such as aetiology, hepatic function, vascular invasion, tumour burden, extrahepatic spread, and AFP levels. Moreover, a group of clinically homogeneous patients included in a study might actually be very heterogeneous biologically. Such heterogeneity could negatively affect the accrual of a homogeneous population in clinical trials of HCC. This concept is also illustrated by the wide range of OS in the placebo arm of second line clinical trials. As a consequence, the statistical power of a clinical trial may be insufficient if a population with a better prognosis is enrolled in placebo arm compared to the population enrolled in previous trials. If the antitumour activity of the experimental drug is insufficiently strong, it may be fairly difficult to achieve an OS improvement. This explanation is consistent with the observation of positive phase II trials and improvements in secondary endpoints in phase III trials (TTP and ORR) that, however, does not translate into an OS benefit.

– *Complex intratumour heterogeneity of HCC*

Recent developments in molecular and genomic technologies enhanced the knowledge of HCC intratumour heterogeneity at morphology, immune phenotypes, and mutational status levels, variously combined with each other. As a result, different cell populations coexist within the same tumour, each with different biomarker expressions and mutational status. Intratumour heterogeneity represents an obstacle to an inclusive classification of HCC and might contribute to the inefficacy of therapy when targeting single molecules of hepatocarcinogenesis. At a molecular level, the identification of "oncogenic drivers" and molecular subclasses remains challenging. More than 30 somatic mutations per tumour have been revealed by extensive genomic studies [67], but the real issue is the ability to distinguish between oncogenic

drivers and passenger mutations [68]. Moreover, even if a driver is found, inhibiting pathways may induce resistant mutations.

Another potential obstacle to the comprehensive knowledge of intratumour heterogeneity is caused by performing molecular analyses on small pieces of tumour, which might underestimate the complexity of tumour genomics.

Selecting patients based on biomarker tissues on a single biopsy might not solve the problem of intratumoural heterogeneity. "Liquid biopsy" is under evaluation, but it has yet to be validated in a clinical setting.

Targets explored might not be true oncogenic drivers in advanced HCC, after sorafenib exposure or drugs used might not be effective inhibitors of that pathway. It is also plausible that combination therapy is needed to exert a significant antineoplastic activity on a certain pathway. In fact, this approach was examined in the phase III CELESTIAL trial that investigated cabozantinib, a multitargeted TKI with doubled activity against both MET and angiogenic pathways in patients with HCC after sorafenib. Cabozantinib showed a significant improvement in median OS, PFS, and RR versus placebo (OS 10.2 vs 8.0 months; PFS 5.2 vs 1.9 months; RR 4.0% vs 0.4%) [11].

2- *Liver-related factors*

– *Hepatic functional reserve*

It is well known that underlying cirrhosis aetiology and liver functional reserve might affect both outcomes and treatment responses. Currently, patients enrolled in clinical trials are stratified according to aetiology as well as Child-Pugh status. Most clinical trials enrol patients with well-preserved liver function (Child-Pugh A). However, it is noteworthy that patients with a score of 5 and those with a score of 6 points have significantly different prognoses. Thus, Child-Pugh scores of 5 and 6 should be used as separate stratification factors.

Liver dysfunction and coexisting cirrhosis may affect drug metabolism so that even in patients with conserved baseline hepatic function, hepatotoxicity can limit the attainment of the optimal maximum tolerated dose. As a consequence, the median treatment duration in clinical trials in which the dosage is reduced due to toxicity might have been insufficient to elucidate a response to this kind of agent.

In a sunitinib phase II trial with a standard dose of 50 mg, 5–10% of the patients enrolled experienced grade 3–4 liver related toxicity, including encephalopathy, hepatorenal syndrome, oesophageal variceal bleeding, ascites, and thrombocytopenia [69]. Almost certainly the inadequate dosing and consequent toxicities contributed to the failure of sunitinib in the phase III trial, which was prematurely terminated due to both futility and safety reasons despite the reduced dosing schedule used (37.5 mg/day) [6]. The frequency and severity of the AEs were comparable to those seen in a phase II study; 18.8% of the patients died due to treatment-related causes, raising concerns regarding an overinterpretation of the phase II results.

Given the peculiar aspects of liver functional reserve in HCC, an important issue has arisen regarding the design of tumour-specific phase I trials including exclusively HCC patients to better establish a maximum tolerated dose (MTD) rather than extrapolate the results of phase I studies including other primary tumours and enrolling patients with normal liver function.

3- *Improper study design*

– *Biopsy requirement*

To date, most clinical trials, especially in the second line setting, do not require fresh tumour biopsy, relying on clinical criteria alone or on previous biopsies. This may represent an obstacle to drug development as it inhibits both prognostic and predictive biomarker detection. For example, in the phase II trial testing tivantinib, a selective c-MET inhibitor, no survival benefit was demonstrated in patients with advanced HCC previously treated with sorafenib; however, a post-hoc subgroup analysis showed that patients with high baseline expression of c-MET on tumour tissue had longer OS (OS 7.2 vs 3.8 months for placebo, HR = 0.38, $p = 0.01$) [70].

– *Enrolment timing*

Studies requiring fresh tumour biopsy with centralised molecular analysis before enrolment might have selected patients with better prognoses. This could be one of the reasons for tivantinib's failure, as the authors explained in the full published paper of the study.

In the METIV-HCC phase III trial, the median time needed to perform a biopsy and obtain the results was 43 days (range 11–406); only patients who remained in a good performance status over that time were enrolled in the trial. Thus, while there might have been screening failures for some patients with MET-high HCC with rapid disease progression, only patients with less aggressive disease might have been included [10]. This "filtering effect" might be the reason why the median OS in the placebo group among patients with MET-high tumours was longer (median 9.1 months) than the median OS in the placebo group (3.8 months) in the phase II trial. Furthermore, this "filtering effect" might have affected enrolment. The patients with more aggressive tumours and with deterioration of liver function or physical status became ineligible, while the patients with less aggressive

tumours were enrolled in the study and eventually had good survival. The patients enrolled might not have benefited from tivantinib because the MET pathway was no longer a driver in their disease progression.

- *Stratification factors*

  Vascular invasion and extrahepatic spread represent important prognostic factors in HCC and both are usually used for patient stratification in clinical trials. Several clinical trials often specified "vascular invasion and/or extrahepatic spread" or "neither vascular invasion nor extrahepatic spread" as allocation factors, thus assigning vascular invasion to the same category as extrahepatic spread, although vascular invasion is a poor prognostic factor for HCC, much more than extrahepatic spread.

  For example, this kind of stratification was used in the BRISK-PS study, a phase III trial comparing brivanib to placebo in patients previously treated with sorafenib. Imbalances in some baseline factors were observed in this study, most notably in AFP and the presence of vascular invasion in favour of the placebo arm. Thus, it can be assumed that the patients with a better prognosis were enrolled in the placebo arm and this condition might explain the failure to detect a difference in OS. Also, an axitinib second line phase II trial used the same stratification criteria for this issue [71]. On the contrary, in the RESORCE study (regorafenib, phase III), which was a positive trial, vascular invasion and extrahepatic spread were used separately as stratification factors [72].

- *Influence of pre-treatment and post-treatment condition*

  Most of the second line trials in HCC evaluated both patients who failed or discontinued sorafenib due to adverse events. The latter group represents up to 12–33% of all the patients enrolled in these studies. However, these two populations differed from each other. In general, the patients who progressed after sorafenib compared to the patients who were intolerant to sorafenib had a relatively greater tumour burden and poorer prognosis. The patients intolerant to sorafenib maintained a relatively stable disease and were more likely to receive subsequent treatments, including locoregional therapy. In support of this argument, a subanalysis of the phase II trial of axitinib in second line revealed that more patients in the placebo arm were intolerant to the first line therapy than in the axitinib arm (25% vs 10%); after excluding the patients who discontinued sorafenib due to adverse events, a significant OS benefit was observed in the axitinib arm [71]. Therefore, clinical trials in a second line setting should enrol only patients progressing to sorafenib, as in the RESORCE trial [72]. Because of the similar chemical structure and toxicity profiles in this trial, patients intolerant to sorafenib were excluded, thereby limiting post-trial treatments. This kind of trial design resulted in a shorter PPS and therefore a greater difference in OS was likely to be demonstrated, as was actually shown in the RESORCE trial.

- *Weak data in early phase trials*

  The clinical success of a new drug largely depends on a rigorous development process based on the demonstration of hypothesised pharmacological effects in early stage trials. Several drugs tested in phase III trials in HCC lacked rigorous development processes and efficacy data often derived from relatively small single-arm phase II studies. In some cases, the engagement of the intended target in humans has not been demonstrated before phase III trials and the biological basis for the expected clinical response has not been well established. It can be challenging to extrapolate efficacy data from phase II trials. Also, in phase III trials, surrogate endpoints such as PFS, TTP, and RR do not always predict OS. In fact, the correlation between tumour progression and survival in HCC may be influenced by the coexistent risk of death derived from liver dysfunction. As previously mentioned, several treatments tested, such as sunitinib and linifanib, have been demonstrated to contribute to hepatic function failure. Thus, it is important to test toxicity in the earlier phases of clinical drug development on a population that accurately reproduce HCC general population features. Preclinical models often cannot reproduce all the features of human tumours. Regarding antiangiogenic pathways, at the time of diagnosis and/or surgery, tumours have already formed new vessels, so studying these mechanisms is a major challenge in the in vivo model.

- *Mechanism of action*

  Most of the agents tested over the last decade in advanced HCC were TKIs with antiangiogenic activity. The large-scale phase III trials testing TKIs showed similar median OS of 9 to 10 months, meaning that the benefit of TKIs in HCC has likely reached a plateau.

  Moreover, the targets explored might not be true oncogenic drivers in advanced HCC after sorafenib exposure or the agents used might not be effective inhibitors of these pathways. In the latter condition, combination therapy might exert a more relevant antitumoural effect on a certain pathway. At the same time, recent insights into the molecular complexity of HCC suggest that a single biomarker might not be enough for the advance of personalised management.

  Combination treatments based on targeted therapies in addition to chemotherapy, radiotherapy, TACE, and dual or multiple targeted therapies have been tested in several clinical trials, albeit one of the hardest obstacles to that approach is the compromised hepatic reserve in most HCC patients [73].

The "multitargeted" approach constitutes the biological rationale for cabozantinib, a multitargeted TKI that inhibits the activity of c-MET and other tyrosine kinases involved in angiogenic pathways. In the CELESTIAL phase III trial comparing cabozantinib with placebo in patients previously treated with sorafenib, a significant improvement in the median OS, PFS, and RR was observed favouring cabozantinib (OS 10.2 vs 8.0 months; PFS 5.2 vs 1.9 months; RR 4.0% vs 0.4%) [11].

## Strengths and weaknesses of REACH trials and ramucirumab development in HCC

Considering the previous explanations, in the REACH-2 design, two main strengths may be assumed to have played a crucial role in the positive result of the trial. First, AFP can be considered a surrogate biomarker for VEGFR-2 expression, thus identifying an approachable way to size the ramucirumab target in vivo. Second, patient enrolment according to elevated AFP may have selected a population with poor prognosis so that the drug efficacy has been emphasised and the statistical power of the OS analysis has been increased to show positive results. However, assessing the global clinical development of ramucirumab in HCC, several weaknesses might be recognised to even consider the positive results of REACH-2, which is unusual.

Before entering the phase III trial in HCC, a significant amount of data on ramucirumab was not obtained, especially regarding the analysis of the predictive biomarkers. Ramucirumab was tested only in a non-HCC-specific phase I trial and a subsequent phase II study in a first line setting with a small sample size. Although a serum biomarker analysis performed parallel to ramucirumab administration in the phase II assessment, the trial did not require mandatory tumour biopsy to emphasise the investigation of the predictive biomarkers and to link the molecular signatures with the therapeutic response. This rush to phase III studies may have contributed to the delay in the discovery of the role of AFP in patient selection and the omission of other potentially predictive biomarkers.

## Discussion

The introduction of next-generation sequencing technologies has elucidated the molecular characterisation of both tumour cell populations and tumour microenvironments. Compared to other cancers, such as breast cancer and NSCLC, in HCC, molecular tumour assessment has not yet become a clinical reality and it is urgently necessary to translate molecular knowledge into precision medicine through the identification of reliable predictive biomarkers.

Patients with advanced HCC represent a distinctive clinical challenge and require a multidisciplinary approach. Clinical decisions should be individualised considering both tumour biological heterogeneity and patient's peculiarities, including the stage of disease, liver function, performance status, comorbidities, and patient preferences [74].

In advanced HCC, despite increasing "statistically" positive phase III trials by few target agents, there is a clear need for therapeutic options with higher levels of evidence and meaningful clinical benefits. In the last EASL HCC guidelines, several unmet needs were highlighted, including surrogate markers for OS, translating molecular features into personalised treatment, and the use of diagnostic biopsy in all cases once a tissue biomarker predicting response is available [3]. Hence, it is expected that forthcoming therapeutic strategies in HCC scenarios will integrate core and subtype-specific transcriptional hallmarks to enhance personalised management [75].

To decrease the likelihood of failure in future studies, a substantial change in the design and conduct of clinical trials is required [76]. In general, future clinical trials should not be static, but should be flexible with adaptive designs to incorporate emerging clinical data as they become available and maintain the pace of advances in cancer molecular characterisation. A proper example of innovative trial conduct could be represented by the seamless transition from a phase I trial to a multicohort expansion phase. This kind of approach, increasingly used in cancer immunotherapy, implies a complex patient recruitment by demanding molecular prescreening. Thus, tumour biopsy might become essential to identify potential responders. Early phase trials might represent the proper field to translate basic scientific discoveries and clinical rationale to assess pharmacodynamics and monitor responses or to detect therapeutic resistance and the underlying mechanisms. Molecular stratification in early phase trials might also decrease the number of patients needed to show an effective response in larger randomised clinical trials.

One major obstacle to the development of a biomarker-driven strategy might be the low frequency of hypothetical predictive biomarkers, which could explain the high rate of screening failure in clinical trials. To overcome this issue and demonstrate meaningful results in clinical trials of targeted agents, enrichment trials and umbrella trials might be established to maximise the benefit of conducting concurrent small-scale "proof of concept" studies in a biomarker-selected population. Establishing umbrella trials is a fairly complex process that requires solid teamwork involving clinicians, ethics committees, pharmaceutical companies, and health regulatory bodies. However, this approach might help patient compliance and acceptance of undergoing a potentially risky biopsy procedure before being enrolled in a clinical trial but with a small chance of accrual.

To overcome the difficulty in obtaining adequate material for molecular analysis since tumour tissue may not be easily accessible in HCC patients, liquid biopsy with the analysis of the

circulating tumour markers (that is, circulating tumour DNA) could represent a non-invasive alternative to profiling molecular changes and would also allow serial monitoring of the evolving genetic and epigenetic modifications in the tumour cells [77].

Finally, since the preservation of health-related quality of life (HR-QoL) is a major treatment goal for patients with advanced cancer, patient-reported outcomes (PROs) should be increasingly included in clinical trial designs, especially in the palliative setting. PROs together with side effect profiles of the treatments now available in the second line setting might also influence the choice of treatment for each patient and costs to well define the real magnitude of the clinical benefits derived by new drugs and their cost-effectiveness. Interestingly, data from a subsequent analysis of the REACH trial showed no worsening of QoL in patients treated with ramucirumab [78].

## Conclusion

To date, the absence of clear predictive biomarker data on the best sequence or combination among all of the approved agents make the second line treatment of HCC an evolving scenario. Thus, parallel to drug development, it is also important to identify predictive biomarkers. In this context, ramucirumab could be considered a very intriguing option for the subgroup of patients with elevated AFP who progressed or were intolerant to first line treatment with sorafenib.

### Compliance with ethical standards

**Conflict of interest** Rodriquenz MG declares that she has no conflict of interest. Sohbani N declares that he has no conflict of interest. Petrioli R declares that he has no conflict of interest. Roviello G declares that he has no conflict of interest.

**Ethical approval** This article does not contain any studies with human participants or animals performed by any of the authors. All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

**Informed consent** For this type of study, formal consent is not required.

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