Biology and significance of alpha-fetoprotein in hepatocellular carcinoma

Peter R. Galle1 | Friedrich Foerster1 | Masatoshi Kudo2 | Stephen L. Chan3 | Josep M. Llovet4,5,6 | Shukui Qin7 | William R. Schelman8 | Sudhakar Chintharlapalli8 | Paolo B. Abada8 | Morris Sherman9 | Andrew X. Zhu10

1Department of Internal Medicine I, University Medical Center of the Johannes Gutenberg University Mainz, Mainz, Germany
2Kindai University, Osaka-Sayama, Japan
3Chinese University of Hong Kong, Honk Kong, China
4Translational Research in Hepatic Oncology, Liver Unit, Institut d’Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Hospital Clinic, Universitat de Barcelona, Barcelona, Spain
5Mount Sinai Liver Cancer Program, Division of Liver Diseases, Tisch Cancer Institute, Icahn School of Medicine at Mount Sinai, New York City, NY, USA
6Institució Catalana de Recerca i Estudis Avançats (ICREA), Barcelona, Spain
7Cancer Center of Bayi Hospital, Nanjing Chinese Medicine University, Nanjing, China
8Eli Lilly and Company, Indianapolis, IN, USA
9Toronto General Hospital, Toronto, ON, Canada
10Massachusetts General Hospital Cancer Center, Harvard Medical Center, Boston, MA, USA

Abstract
Hepatocellular carcinoma (HCC) is one of the most common causes of cancer-related deaths globally due, in part, to the majority of patients being diagnosed with intermediate or advanced stage disease. Our increased understanding of the heterogeneous molecular pathogenesis of HCC has led to significant developments in novel targeted therapies. Despite these advances, there remains a high unmet need for new treatment options. HCC is a complex disease with multiple pathogenic mechanisms caused by a variety of risk factors, making it difficult to characterize with a single biomarker. In fact, numerous biomarkers have been studied in HCC, but alpha-fetoprotein (AFP) remains the most widely used and accepted serum marker since its discovery over 60 years ago. This review summarizes the most relevant studies associated with the regulation of AFP at the gene and protein levels; the pathophysiology of AFP as a pro-proliferative protein; and the correlation of AFP with molecular HCC subclasses, the vascular endothelial growth factor pathway and angiogenesis. Also described are...
the historical and current uses of AFP for screening and surveillance, diagnosis, its utility as a prognostic and predictive biomarker and its role as a tumour antigen in HCC. Taken together, these data demonstrate the relevance of AFP for patients with HCC and identify several remaining questions that will benefit from future research.

KEYWORDS
alpha-fetoprotein, biomarkers, hepatocellular carcinoma

1 | INTRODUCTION

Hepatocellular carcinoma (HCC), the leading primary malignancy of the liver, is one of the most common cancers globally and results in significant health-related problems, making it the third most frequent cause of cancer-related deaths.1-4 HCC is difficult to treat and manage as a result of late detection, high rates of tumour recurrence, resistance to classical chemotherapy and radiotherapy, and notable molecular heterogeneity. While early stage disease is associated with 5-year survival rates from 40% to 70%, most patients are diagnosed with intermediate or advanced stage disease for which curative therapies are no longer an option.5

Despite recent advances in the understanding of the molecular pathogenesis of HCC leading to the development of new approved therapies, treatment options for advanced stage disease remain limited. Until recently, sorafenib, an oral, systemic, multikinase inhibitor, was the only approved treatment for advanced HCC and demonstrated a modest improvement in survival with an increased incidence of adverse events when compared with placebo.5 While several phase 3 studies of targeted therapies failed to show improvement over sorafenib in the first-line setting, a few targeted therapies have recently been approved in different settings including lenvatinib in the first-line setting, and regorafenib and cabozantinib following progression on first-line therapy.6 Recent exploratory analyses suggest median overall survival (OS) of up to 26 months may be achieved with sequential treatments,7,8 but will require prospective studies to confirm. Although there have initially been promising clinical results with immune checkpoint inhibitors, their role in this disease remains uncertain. Despite these advances, the current prognosis for advanced disease remains dismal with median OS ranging from 7.3 to 13.6 months, median progression-free survival (PFS) from 3.1 to 7.4 months and objective response rates from 2% to 24%.6

Current research efforts are aimed at identifying specific HCC patients who will benefit from new therapies and several prognostic or predictive biomarkers are being used or studied to improve outcomes. The most commonly used biomarker for HCC is serum alpha-fetoprotein (AFP).9 Historically, AFP has been used for screening and diagnosing HCC, predicting prognosis and monitoring response to treatment. However, its use in some settings has been controversial, particularly with respect to surveillance and diagnosis.

Key points
• Alpha-fetoprotein (AFP) is the most widely accepted serum biomarker used in the management of hepatocellular carcinoma (HCC).
• The regulation and pathophysiology of AFP informs the basis for its clinical relevance in the context of HCC.
• Several first-line and second-line trials show the prognostic effect of AFP, and now ramucirumab studies with enriched patient populations have demonstrated its utility as a predictive marker for antiangiogenic treatment.
• Growing evidence suggests pretransplant AFP is a useful prognostic marker for selecting liver transplant candidates and assessing risk of recurrence.
• Future clinical studies will strengthen our understanding of this important biomarker for patients with HCC.

2 | BIOMARKERS IN HCC

Although the search for other diagnostic, prognostic or predictive biomarkers for HCC has been extensive, AFP remains the most commonly used biomarker in HCC. For patients who are at risk of developing HCC, additional biomarkers could detect the cancer at an earlier, potentially curative stage. For those with a diagnosis of HCC, novel biomarkers could identify biochemical or clinical factors indicative of clinical outcomes and/or measure of disease burden (‘prognostic’). Likewise, they could also be correlated with tumour response and clinical outcomes to specific therapies (‘predictive’). There are several factors that determine the effectiveness of a biomarker, including those attributable to the type of tumour, its prevalence in the investigated population and the availability of an effective therapeutic regimen.

A growing body of literature describes potential predictive and prognostic biomarkers that may inform diagnosis and treatment of HCC. For early detection and/or diagnosis of HCC, oncofetal antigens, proteoglycans, enzymes and isoenzymes, such as the fucosylated fraction of AFP (AFP-L3), des-gamma carboxyprothrombin (DCP; also known as prothrombin induced by vitamin K absence or antagonism II), versican and glypican 3, have been evaluated.10-12 Clinical features related to tumour stage and treatment have also been characterized, including Barcelona Clinic Liver Cancer (BCLC)
stage and macroscopic vascular invasion. Several retrospective studies evaluating laboratory and clinical findings during treatment have also identified potential predictive markers of response or resistance to therapy. During treatment with sorafenib, hand-foot skin reaction, hypertension or diarrhoea have been associated with clinical benefits. Likewise, low neutrophil to lymphocyte ratio, aetiology, extrahepatic spread, high s-c-KIT, low baseline hepatocyte growth factor concentration, FGF3/FGF4 amplification and rare vascular endothelial growth factor-A (VEGF-A) amplification have also been associated with improved response to sorafenib. Conversely, elevated expression of VEGF-A, K19, CD133, epithelial cell adhesion molecule (EpCAM) or serglycin as well as single nucleotide polymorphisms and angiopoietin-2 levels in patients receiving sorafenib have been associated with poor prognosis. An exploratory analysis of the RESORCE trial suggests that decreased expression of lectin-like oxidized LDL receptor 1, Ang1, cystatin-B, latency-associated peptide TGF-β1 or macrophage inflammatory protein 1α may be predictive of the OS and time to progression treatment benefit observed from regorafenib, but these findings require prospective studies to confirm. In a large, phase 3, randomized, double-blind, placebo-controlled study, tivantinib did not improve OS in patients with high MET expression on tumour cells and advanced HCC that had progressed after sorafenib-based therapy, despite the anticipated predictive value of MET identified in a phase 2 study. In the large, phase 3, randomized trial REFLECT, lenvatinib demonstrated noninferiority to sorafenib in OS and improved response rates in patients with unresectable HCC in the first-line setting. Final biomarker analysis of the REFLECT trial suggested that higher baseline levels of VEGF, ANG2 and FGF21 were associated with worse OS in both treatment arms. However, longer OS for lenvatinib vs sorafenib was observed in patients with high baseline levels of FGF21, suggesting FGF21 could be predictive for reduced OS with sorafenib compared to lenvatinib.

Despite the identification of candidate biomarkers, AFP is still the most widely accepted and used serum marker in HCC. The aim of this review is to understand the significance and clinical relevance of AFP as a biomarker and tumour antigen in HCC.

3 | HISTORY AND PHYSIOLOGY OF ALPHA-FETOPROTEIN

Alpha-fetoprotein is a 70 kD glycoprotein that is produced by the fetal liver and yolk sac during the first trimester of pregnancy. In 1956, it was identified from a protein fraction detected in human fetal serum that was not detected in adult serum (Figure 1). The isolated protein was subsequently termed AFP, and acts as the fetal equivalent of serum albumin. In normal physiology, AFP declines rapidly after birth and remains at low levels over the entire lifespan (Figure 2). A study conducted in the late 1980s concluded that the absolute size of the fetus as well as gestational age might play a significant role in determining maternal and fetal AFP concentrations, and that there is a significant correlation between maternal, cord arterial and venous AFP. Likewise, low levels of maternal serum AFP during the second trimester were subsequently shown to be associated with a very low risk of preterm birth, pre-eclampsia and placental complications, and vice versa. Owing to the variability in absolute AFP concentrations in healthy newborns, the kinetics of AFP declining during the neonatal period is commonly followed in clinical practice. The AFP gene is one of the four members of the albumin gene family localized in a tandem arrangement to form a multigene cluster, and there are three major isoforms defined by their affinity for the lectin Lens culinaris agglutinin (AFP-L1, AFP-L2 and AFP-L3) that
are found in varying amounts in different physiological or pathological conditions.\(^4\) AFP expression is primarily regulated at the transcriptional level (Figure 3), and the gene has an upstream regulatory region that consists of a tissue-specific promoter, three independent enhancers and two silencer regions, which may be involved in the decreased AFP gene expression in adult livers.\(^29\) Following fetal liver development, the AFP enhancers are normally blocked from the gene promoter and instead act to maintain albumin gene transcription into adulthood.\(^30\)

Alpha-fetoprotein was recognized as the first oncofetal biomarker after it was identified in the serum of patients with HCC and undifferentiated teratoma. The test for AFP was the first serologic assay used for the detection and clinical follow-up of patients with HCC.\(^31\) A large number of clinical studies have investigated AFP, predominantly in patients with chronic liver diseases.\(^31,32\)

4 | PATHOPHYSIOLOGY

4.1 | Mechanisms of AFP overexpression

Although expressed in 60% to 80% of HCC, genetic regulation of AFP is complex and has not been fully characterized.\(^33\) Based largely on preclinical studies, AFP expression appears to be suppressed at the promoter and at two enhancers by corepressors and methylated histones in adult cells.\(^34\) Repression is mediated, in part, by zinc-fingers and homeoboxes 2 (ZHX2) and BTB domain containing 20 (ZBTB20).\(^35,36\) In HCC, hypermethylation and resultant silencing of ZHX2 was found to be a potential mechanism of AFP overexpression, and overexpression of ZHX2 inhibited the AFP synthesis and secretion.\(^35\) Further, deregulation of pathways impacting ZBTB20 expression in the liver, which may be through the microRNA122 pathway, was also shown to contribute to AFP overexpression and HCC tumour aggressiveness.\(^37\) Specifically, microRNA122 regulated the levels of ZBTB20 via a complex pathway involving the Cut homeobox 1 (CUX1), a protein that regulates cell motility and invasion.\(^37\)

Despite some understanding of the regulation of AFP expression based on preclinical work, the limited clinical observations in patients with HCC have been less clear. A small study in Peruvian patients with HCC demonstrated a contrasting relationship between the two repressors in which ZBTB20 was downregulated whereas ZHX2 was enhanced. However, both repressors appeared to contribute to AFP overexpression.\(^38\) To add complexity, other studies have identified additional effects of cytokine signalling, such as TGF-β, on AFP expression,\(^39\) which is only further complicated by the well-known fact that TGF-β also has distinct roles in tumour inhibition vs tumour progression depending on the stage of disease.\(^40\) Despite progress to date, additional research is clearly needed to better characterize the regulation of AFP in HCC.

4.2 | Role as pro-proliferative protein

AFP may regulate the growth of neoplastic and normal cells by several mechanisms that include apoptotic regulation and cytoplasmic signalling modulation. Although increasing evidence suggests that AFP may regulate the growth of tumour cells, the specific mechanism for its growth-promoting activity is unclear. In HL-60 cells and HepG2 cells, AFP was shown to protect against apoptosis induced by various factors.\(^41,42\) Since tumour proliferation by AFP was found to be dependent on the cyclic AMP-protein kinase A pathway and the initiation of Ca2+ influx, a possible explanation could be that increases in intracellular Ca2+ from AFP-induced Ca2+ influx results in increased DNA synthesis and tumour proliferation mediated

**FIGURE 3** Concordant dysregulated expression of alpha-fetoprotein and other genes in the fetal liver, a healthy adult, or in a patient with hepatocellular carcinoma
by intracellular cAMP and protease A activity. In vitro, AFP also affects the function of caspase-3 via indirect interaction with the X-linked inhibitor of the apoptosis protein, XIAP, and the cellular inhibitor of apoptosis protein, cIAP-2. As a result, AFP may play a critical role in the inhibition of the apoptotic signal transduction mediated by caspase-3.

Several studies also suggest that tumour growth results from AFP-mediated suppression of the antitumour immune response. AFP was shown to interact with macrophages and to decrease their phagocytic activity and la antigen expression. In addition, AFP inhibits the activity of natural killer cells, reduces proliferation of T-lymphocytes and promotes the activity of T-suppressor cells (reviewed in Terentiev et al).

Detailed mechanisms regarding the upregulation of cell proliferation and tumour growth by AFP remain unknown. However, studies have shown that AFP binds to specific receptors located on the surface of normal and tumour cells, and the presence of AFP and its receptor in human placenta suggests a possible receptor-mediated mechanism for placental transport of AFP between the fetal and maternal circulations. AFP may influence the delivery of fatty acids to proliferating cells that require increased energy supply and intermediate products of β-oxidation of fatty acids. In reaction to lymphocytic blast transformation, AFP and the heptapeptide AFP might also cause a moderate stimulation of proliferation of lymphocytes and inhibition of proliferation of PHA-activated lymphocytes at concentrations of 10⁻¹ to 10⁻⁸ M. Since the heptapeptide AFP inhibits proliferation of lymphocytes in a dose-dependent manner from patients with acute and chronic lymphocytic leukaemia with low sensitivity to antitumour agents, AFP may be a biologically active ligand on certain human cells. Taken together, these data suggest that AFP may have dual regulatory effects on cell proliferation and tissue growth through both stimulatory and inhibitory effects.

4.3 | Correlation with molecular HCC classes

Hepatocellular carcinoma is a heterogeneous tumour on the macroscopic, histopathological and molecular level. Molecular aberrations have been identified that allow the subclassification of HCC by molecular and clinical characteristics. In addition, different transcriptomic subclasses have been described and linked to histological subtypes. Through a global transcriptome analysis of 120 HCC tumours, Boyault et al described six robust subgroups of HCC tumours (G1-G6) that are associated with clinical and genetic characteristics and reflect the large heterogeneity of HCC tumours. Hepatitis B virus infection was the primary clinical determinant of class identification for the G1 and G2 subgroups. G3-G6 tumours were more related to hepatitis C virus infection and alcohol abuse. Other predominant determinants included genetic and epigenetic alterations such as chromosome instability, CTNNB1 and TP53 mutations, and parental imprinting. A meta-analysis of gene expression profiles from 603 HCC patient samples identified three robust HCC subclasses, S1-S3, associated with various parameters that included tumour size, degree of cellular differentiation and serum AFP levels. The G1, G2 and G3 subclasses, which are characterized by high proliferation and chromosomal instability and the S2 subclass, which is linked to large tumours, have been shown to be associated with high AFP serum levels (G1-G3: AFP > 100 ng/mL; P < .001, and S2: median AFP = 171 ng/mL; P < .001). Furthermore, AFP and EpCAM expression have been used to divide HCC patients into prognostic molecular subclasses. These results suggest AFP may be useful for detecting a molecular subclass of HCC. However, translating this knowledge into clinical practice will require further research.

4.4 | AFP, VEGF and angiogenesis

Hepatocellular carcinomas are highly vascular, and their growth is dependent on angiogenesis. The therapeutic effect of transarterial (chemo-) embolization and agents with antiangiogenic properties such as sorafenib support the vascular nature of these tumours. High serum and tissue VEGF levels are associated with poor disease-free and OS in HCC. In addition, VEGF has been reported as a prognostic biomarker in advanced HCC. AFP and EpCAM, a hepatic stem cell expression marker, have also been implicated in the prognosis of HCC patients and HCC patients with high AFP serum levels (>300 ng/mL) and positive staining for EpCAM have been shown to have significantly higher VEGF tissue expression and microvessel density. AFP has also been specifically evaluated as a predictor of efficacy to antiangiogenic therapy in HCC. There is a paucity of literature demonstrating a mechanistic link between AFP and angiogenesis. However, emerging data suggest crosstalk of AFP and VEGF signalling cascades. Genetic mechanisms do exist that may concordantly dysregulate the expression of AFP and several other known and unknown genes involved in angiogenesis during HCC progression. Likewise, silencing of AFP has been shown to inhibit VEGF production in HCC cells in vitro. Taken together, these findings are consistent with AFP expression being associated with potentially more angiogenic tumours and, as described above, may denote particular subclasses of HCC.

5 | CLINICAL RELEVANCE OF AFP IN HCC

5.1 | AFP for defining patients at risk of HCC development: screening and surveillance

Early detection of HCC may improve outcomes, and persistently elevated AFP levels have been identified as a risk factor for development of HCC and could potentially help define at-risk populations. The use of AFP alone to screen the general population for HCC has proven controversial since elevated AFP levels may occur in other benign liver conditions. Because the prevalence of HCC in the general population is low and the positive predictive value (PPV) of AFP is poor (at 5% prevalence, its PPV has been calculated to be 25.1% using a cut-off of 20 ng/mL), AFP alone is not recommended as a screening tool to identify individuals with an increased HCC risk in the general population.
For patients with risk factors for developing HCC, surveillance with AFP is suboptimal as AFP levels are normal (≤20 ng/mL) in about 30% to 40% of patients with HCC and may also be elevated because of nontumour-related causes, such as chronic viral hepatitis, leading to reported sensitivities of 58% to 68% and specificities of 80% to 94% (20 ng/mL cut-off). Moreover, AFP is particularly insensitive for the detection of small HCC, which limits the usefulness of screening in this important setting. Of note, it has been shown that antiviral therapy can reduce AFP baseline levels, improving the diagnostic accuracy of AFP in patients with chronic hepatitis B. In turn, delayed AFP response to antiviral therapy may serve as an indicator of increased HCC risk.

It has been debated whether applying a different AFP cut-off value might turn AFP into a suitable surveillance parameter. However, owing to the inverse relationship of sensitivity and specificity, a meta-analysis of studies with varying AFP thresholds has clarified that a different AFP cut-off value results in either unacceptably high false-positives or false-negatives. A different strategy of using algorithms based on dynamic levels of AFP in addition to other clinical and biological factors may help increase the usefulness of AFP levels in surveillance. El-Serag et al developed an algorithm based on AFP levels, platelet count, alanine aminotransferase and age that increased the predictive value for identifying at-risk patients more likely to develop HCC within 6 months and could easily be integrated into clinical practice. A fully Bayesian univariate screening algorithm with longitudinal AFP and DCP also detected at least one positive screen in 89.5% of HCC cases with a 10% false-positive rate using control patients from the HALT-C trial.

GivenAFP’s performance as a screening parameter in HCC surveillance, AFP alone is not sufficient for the diagnosis of HCC and, therefore, contemporary imaging techniques (for the most part, CT or MRI) are used for screening. However, AFP does have a role as a supplemental test in the clinical setting when the imaging findings are inconclusive or cannot be clearly differentiated from other kinds of liver cancer such as cholangiocarcinoma. A large, randomized, controlled trial that assessed the effect of screening on HCC mortality in at-risk patients determined that ultrasound and serum AFP testing every 6 months reduced HCC mortality by 37% despite poor compliance with the program. Likewise, a surveillance program study that enrolled patients with Child A/B cirrhosis found that ultrasound screening combined with AFP significantly increased the sensitivity of ultrasound screens from 43.9% to 90.2%. Consequently, practice guidelines currently recommend liver ultrasound with or without AFP levels as a screening method for patients at risk for HCC. Guidelines from Japan recommend inclusion of AFP levels in surveillance programs. The biomarker-combined Japan Integrated Staging (bm-JIS) score includes AFP, AFP-L3 and DCP specific for HCC to stratify patients and predict prognosis. In the United States, guidelines suggest that serum AFP levels are optional for use in conjunction with ultrasound for screening at-risk populations. European guidelines have recently reported that adding AFP measurements to ultrasound as the predominant surveillance tool leads to a modest increase of 6% to 8% in detected HCCs.

5.2 | AFP as a prognostic factor in HCC

Elevated AFP serum levels are associated with a poorer prognosis in HCC patients, and serum AFP concentrations ≥400 ng/mL consistently denote poorer prognosis in different clinical settings. AFP has been proven to be a valuable addition to models used for identifying the best candidates for liver transplantation (also in the living donor liver transplantation setting); additionally, high AFP serum levels have been shown to predict the risk of tumour recurrence after hepatic resection, the risk of drop-out while on the waiting list for liver transplantation and the risk of tumour recurrence after liver transplantation (Table 1). Recently, the combination of pretransplant AFP (with a cut-off of 200 ng/mL) and 18F-fluorodeoxyglucose positron emission tomography has been reported to be superior in predicting 5-year disease-free survival rates in comparison with the traditional Milan criteria.

The rate of increase of AFP before liver transplantation (increase in AFP >50 ng/mL or >15 ng/mL per month) has been shown to be a predictor of HCC recurrence and to be associated with worse survival after transplantation. Interestingly, successful downstaging of AFP levels ≤400 ng/mL before transplantation resulted in significantly better survival, compared with patients who failed to have a reduction in AFP to ≤400 ng/mL (P < .001), and an equivalent survival compared with patients whose AFP had always been ≤400 ng/mL. A similar decrease in recurrence after transplantation has been reported in patients who were bridged with locoregional therapy and whose pretransplant AFP was lowered to ≤13 ng/mL. Therefore, it has been suggested to include AFP levels in the selection of patients for inclusion on liver transplantation waiting lists.

In the nonsurgical setting, pre-intervention AFP levels have been shown to predict survival prognosis with locoregional therapies as well as with sorafenib, lenvatinib, regorafenib, cabozantinib and ramucirumab. Two studies evaluating radiofrequency ablation (RFA) for first-line treatment of HCC in patients with Child-Pugh A/B cirrhosis demonstrated 5-year OS rates of 68% to 76%. Univariate and multivariate analysis of prognostic factors associated with survival showed high baseline AFP levels was associated with HCC recurrence and poor prognosis. Transcatheter arterial chemoembolization (TACE) with lipiodol used as first-line treatment for 4966 Japanese patients with HCC demonstrated a 34% 5-year survival rate, and AFP was among several predictive factors identified in multivariate analysis.

In patients with advanced HCC, the prognostic value of elevated baseline serum AFP levels has been reinforced in several recently completed phase 3 trials. The association of elevated AFP concentrations with poor prognosis has been consistently demonstrated in these trials including SHARP, REFLECT, RESORCE, CELESTIAL and REACH. Owing to the clear prognostic significance of AFP in the advanced setting, baseline AFP concentration has been increasingly used as a stratification factor in phase
| Study | N   | Details regarding AFP                                                                 | Survival rate/Recurrence risk¹ |
|-------|-----|---------------------------------------------------------------------------------------|--------------------------------|
| Ikai et al 2004⁸⁵ | 12118 | Preoperative serum AFP: <20 ng/mL, 21-200 ng/mL, 201-1000 ng/mL, 1001-10000 ng/mL, ≥10001 ng/mL | 3-year/5-year survival rate: 77.1%/61.5%, 67.2%/47.0%, 58.7%/41.5%, 52.1%/37.7%, 40.3%/33.1% |
| Yang et al 2007⁸⁶ (Seoul criteria) | 63 | Last AFP (≤20; 20.1-200; 200.1-1000; >1000 ng/mL) | 3-year OS: Score 3-6 (transplantable): 79.0%, Score 7-12 (nontransplantable): 38% 3-year DFS: Score 3-6: 87.0%, Score 7-12: 31% |
| Kwon et al 2007⁸⁷ (SMC criteria) | 139 | Last AFP ≤ 400 ng/mL | 5-year OS: In criteria: 86.8%, Outside criteria: 23.3% 5-year DFS: In criteria: 88.4%, Outside criteria: 42.1% |
| Zheng et al 2008⁸⁸ (Hangzhou criteria) | 195 | Last AFP ≤400 ng/mL | 5-year OS: In criteria: 70.7%, Outside criteria: 18.9% 5-year DFS: In criteria: 62.4%, Outside criteria: 4.7% |
| Ravaiolì et al 2008⁸⁹ (Bologna criteria for downstaging) | 48 | AFP remained at <400 ng/mL during waiting time | 3-year OS: 72% Recurrence rate: 18.8% 3-year DFS: 71% |
| Tosò et al 2015⁹⁰ (TTV/AFP model) | 233 | AFP ≤400 ng/mL | Within Milan - 4-year OS: 78.7% - 4-year DFS: 77.9% Beyond Milan Criteria and TTV/ AFP-in - 4-year OS: 74.6% - 4-year DFS: 68.0% |
| Vibert et al 2010⁹¹ | 153 | Preoperative AFP: >15 μg/L per month (progression) ≤15 μg/L per month (nonprogression) | 5-year OS/RFS 54%/47% 77%/74% |
| Merani et al 2011⁹² | 6817 | AFP >400 ng/mL downstaged to AFP ≤400 ng/mL AFP >400 ng/mL failed to reduce to ≤400 ng/mL AFP stable at ≤400 ng/mL | Intent-to-treat survival at 3 y: 81% 48% 74% |
| Duvoüx et al 2012⁹³ (AFP model) | 435 | log₁₀ AFP, (simplified version: low-risk pts: AFP ≤100 and 100-1000; High-risk pts: AFP >1000 ng/mL) | 5-year OS: Low-risk: 69.9% High-risk: 40.8% 5-year recurrence rate: Low-risk: 13.4% High-risk: 45.3% |
| Lai et al 2012⁹⁴ (AFP-TTD criteria) | 158 | Last AFP ≤400 ng/mL | Recurrence rate (median follow-up 43 mo): In criteria: 4.9%, Outside criteria: 33.0% |
### TABLE 1 (Continued)

| Study                                      | N   | Details regarding AFP                                                                 | Survival rate/Recurrence risk<sup>a</sup>                                                                 |
|--------------------------------------------|-----|---------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------|
| Vitale et al 2014<sup>95</sup> (Italian transplant benefit model) | 4399 | AFP (≤100; 100–1000; >1000 ng/mL)                                                      | Equation producing a numerical score that matches HCC patients with non-HCC patients: 1.27* MELD - 0.051 logAFP + 4.59 |
| Lai et al 2016<sup>96</sup> (TRAIN score)   | 179 | AFP slope ≥15 ng/mL/month                                                               | ITT 5-year survival:                                                                                   |
| Sapisochin et al 2016<sup>97</sup> (extended Toronto criteria) | 588 | AFPL ≥500 ng/mL; AFPL ≤500 ng/mL                                                       | Actuarial patient survival:                                                                            |
| Halazun et al 2017<sup>98</sup> (pre-MORAL score) | 339 | Maximum AFP from HCC diagnosis to LT >200 ng/mL                                         | 5-year RFS:                                                                                             |
| Lai et al 2017<sup>99</sup> (EurHeCaLT transplant benefit model) | 2103 | Last AFP ≥1000 ng/mL vs <1000 ng/mL                                                    | ITT transplant benefit (months): 6.8 mo vs 25.4 mo                                                   |
| Mazzaferrro et al 2018<sup>100</sup> (Metroticket 2.0 model) | 341 | Pretransplant AFP (<200; 200–400; 400–1000 vs >1000 ng/mL)                             | 5-year OS: Within criteria of 79.7% vs beyond criteria of 51.2% (with a tumour-specific survival of 93.5% within vs 55.6% beyond)  |
| Risk of tumour recurrence                  |     |                                                                                        |                                                                                                          |
| Imamura et al 2003<sup>101</sup>          | 249 | Preoperative AFP ≥32 ng/mL                                                             | Associated with recurrence within 2y (HR: 1.83, 95% CI: 1.25, 2.68)                                    |
| Han et al 2007<sup>102</sup>              | 48  | Preoperative AFP slope: >50 µg/L per month ≤50 µg/L per month                          | One-year RFS: 40%                                                                                      |
| Grat et al 2016<sup>103</sup>             | 146 | AFP persistently <100 ng/mL Initially high AFP dropped to <100 ng/mL                   | 5-year RFS: 97.3%                                                                                      |
| Piñero et al 2016<sup>104</sup>           | 323 | AFPL: ≤100 ng/mL; 101–1000 ng/mL; >1000 ng/mL                                         | 5-year incidence of recurrence: 11.1%                                                                    |
| Notarpaolo et al 2017<sup>105</sup>       | 574 | Last AFP before LT: ≤100 ng/mL; 100–1000 ng/mL; >1000 ng/mL                            | 5-year risk of recurrence: 13.0%                                                                        |
| Mehta et al 2018<sup>106</sup> (RETREAT score) | 3276| Preoperative AFP (ng/mL): 0–20; 21–99; 100–999; ≥1000 ng/mL                            | 3-year recurrence risk:                                                                                |

<sup>a</sup>Scoring systems used in multiple publications for selecting liver transplant candidates or for prognosis may involve AFP as well as other disease-related criteria not specifically listed in the table for the sake of brevity. Please refer to the original publication for additional details.

Abbreviations: AFP, alpha-fetoprotein; AFPL, AFP at listing; DFS, disease-free survival; EurHeCaLT, European Hepatocellular Cancer Liver Transplant; ITT, intent-to-treat; LT, liver transplant; MELD, model for end-stage liver disease; MORAL, Model of recurrence after liver transplant; OS, overall survival; RETREAT, risk estimation of tumour recurrence after transplant; RFS, recurrence-free survival; TRAIN, time-radiological-response-alpha-fetoprotein-inflammation; TTD, total tumour diameter; TTV, total tumour volume.
3 clinical trial design in advanced HCC. Since these treatment modalities are commonly repeated or administered over time in the same patient, in contrast to surgery, it makes sense to consider the dynamic in AFP levels during a treatment course. Generally, the response to locoregional or systemic treatment is assessed by radiological imaging techniques using the RECIST version 1.1 or mRECIST criteria. However, serial serum AFP measurements during the course of HCC treatment are common in clinical practice based on the hypothesis that AFP reflects tumour activity and burden, and can be assessed much more frequently. There have been a number of studies validating the utility of serial AFP measurements to monitor treatment response to locoregional therapies, and a reduction in the serial trend of AFP has been shown to be a marker of response to locoregional therapies including RFA, TACE or SIRT. However, the advantage of using AFP over radiographic assessments is still unclear.

For systemic therapy, initial data from patients undergoing cytotoxic chemotherapy demonstrated that AFP response was a favourable prognosticator. Similar findings were subsequently observed in patients undergoing current standard systemic therapy including sorafenib, sorafenib in combination with TACE, cabozantanib monotherapy or ramucirumab monotherapy in the second-line setting when compared with placebo. The definition of AFP response varies among different studies and treatment modalities. With respect to antiangiogenic treatment, an early AFP response (≥20% decrease from baseline after 4 weeks of treatment in patients with elevated AFP) has been associated with significantly improved response and survival. The role of AFP in monitoring treatment response to check-point immunotherapy is less clear. Case studies have suggested that downtrending AFP levels were associated with radiological response; however, validation in more robust clinical studies is required. Overall, there is evidence that the AFP dynamic reflects response to treatment well, and response assessment by serial AFP measurements should be included in future clinical trials to better characterize the role of AFP monitoring during treatment.

### 5.2.1 Role of AFP in prognostic scoring systems

Among the more comprehensive HCC staging systems, five (three European and two Asian) have been broadly tested, some of which include biomarkers (AFP, AFP-L3 and DCP) as parameters to refine the staging system (Table 2). The French classification (GRETCH), Cancer of the Liver Italian Program (CLIP) classification and the BCLC staging system are the European staging systems. The Chinese University Prognostic Index (CUPI score) and the Japan Integrated Staging (JIS) system are two Asian staging systems.

Two scores that include AFP as a parameter have been proposed. The BALAD score, which was intended as a staging system that is entirely based on serological markers and includes bilirubin, albumin, AFP-L3 and DCP, were also used to refine the JIS system (bm-JIS), resulting in superior stratification ability and better survival prediction in comparison with the conventional JIS score. The BALAD system has been validated in non-Japanese populations. Despite these considerable achievements, both scores have not yet been widely implemented and await confirmation in multicenter, international studies.

### 5.3 Role of AFP as a predictive marker for patient selection in HCC

Baseline levels of serum AFP in patients with advanced HCC may help identify patients who will benefit most from molecularly targeted treatments. Until recently, studies that have identified predictive biomarkers for responsiveness to treatment for patients with HCC have been scarce. In REACH, a global, randomized, double-blind placebo-controlled, phase 3 study, the efficacy and safety of single-agent ramucirumab was evaluated for patients with advanced HCC following first-line sorafenib (Clinicaltrials.gov identifier NCT01140347). Although REACH did not meet the primary objective of improved OS in the ramucirumab arm, an improvement in OS was observed in a prespecified subgroup of patients (n = 250) with baseline serum AFP ≥400 ng/mL treated with ramucirumab compared with placebo (median OS 7.8 vs 4.2 months respectively; hazard ratio [HR] 0.67, 95% confidence interval [CI] 0.51, 0.90; P = .006). This analysis supported elevated AFP as a marker for poor prognosis in advanced HCC; a separate study, discussed below, confirmed the predictive utility of enriching a study population treated with ramucirumab by baseline AFP levels.

Based on the results from the REACH study, single-agent ramucirumab was evaluated in the second-line treatment of HCC patients with an elevated AFP level (≥400 ng/mL) in a phase 3 study (REACH-2; NCT02435433). Ramucirumab significantly improved OS (median OS 8.5 months vs 7.3 months placebo; HR 0.71, 95% CI 0.53, 0.95; P = .0199) and PFS (median PFS 2.8 months vs 1.6 months for placebo; HR 0.452, 95% CI 0.34, 0.60; P < .0001). The objective response rate was 5% for ramucirumab vs 1% for placebo (P = .1697) and the disease control rate was 59.9% for ramucirumab vs 38.9% for placebo (P = .0006). Grade ≥ 3 adverse events occurring in ≥5% of patients in the ramucirumab arm were hypertension (13% ramucirumab, 5% placebo) and hyponatremia (6%, 0%). This was the first phase 3 study validating a biomarker-enriched patient population with advanced HCC.

Evaluation and validation of predictive biomarkers for selected patient subgroups in early clinical trial settings seem intuitive and might avoid trial failure in later stages of clinical development. Clinical trials in progress that limit enrolment of patients with AFP-expressing tumours include an open-label, single arm, phase 1b trial of avelumab plus axitinib for first-line treatment of patients with advanced HCC expressing AFP (≥400 ng/mL) (Clinicaltrials.gov identifier NCT03289533) and a phase 1 open-label study evaluating the safety and efficacy of ET1402L1-CAR T cells that target AFP...
### TABLE 2  Hepatocellular staging systems

| Study       | Tumour markers | Lab chemistry | Clinical parameters | Tumour characteristics |
|-------------|----------------|---------------|---------------------|------------------------|
|             | AFP | AFP-L3 | DCP | Albumin | Bili-rubin | Urea | ALP | Ascites | Child-Pugh | PST | Symptomatic | Tumour size | Tumour morph. | Tumour count | Vasc. invas. | Extrah. spread | TNM |
| bm-JIS63    | X   | X     | X   |         |          |      |     |         |          |     |            |            |                |                |                |                |                |        |        |
| GRETCH128   | X   |       |     |         |          |      |     |         |          |     |            |            |                |                |                |                |        |        |
| CLIP129     | X   |       |     |         |          |      |     |         |          |     |            |            |                |                |                |                |        |        |
| BCLC130     |     |       |     |         |          |      |     |         |          |     |            |            |                |                |                |                |        |        |
| CUP131      | X   |       |     |         |          |      |     |         |          |     |            |            |                |                |                |                |        |        |
| JIS132      |     |       |     |         |          |      |     |         |          |     |            |            |                |                |                |                |        |        |
| BALAD134    | X   | X     | X   |         |          |      |     |         |          |     |            |            |                |                |                |                |        |        |
| Okuda135    |     | X     | X   |         |          |      |     |         |          |     |            |            |                |                |                |                |        |        |
| Tokyo136    |     | X     | X   |         |          |      |     |         |          |     |            |            |                |                |                |                |        |        |
| ALCPS137    | X   |       |     |         |          |      |     |         |          |     |            |            |                |                |                |                |        |        |
| CIS138      | X   |       |     |         |          |      |     |         |          |     |            |            |                |                |                |                |        |        |
| TIL139      | X   |       |     |         |          |      |     |         |          |     |            |            |                |                |                |                |        |        |
| HKLC140     |     |       |     |         |          |      |     |         |          |     |            |            |                |                |                |                |        |        |

Abbreviations: ALP, alkaline phosphatase; ALCPS, advanced liver cancer prognostic system; BCLC, Barcelona Clinic Liver Cancer Classification; bm-JIS, biomarker-Japan Integrated Staging; CIS, China Integrated Score; CLIP, Cancer of the Liver Italian Program Score; CUP, Chinese University Prognostic Index; DCP, Des-gamma-carboxy prothrombin; Extrah. Spread, extrahepatic spread; HKLC, Hong Kong Liver Cancer classification; Morph, morphology; PST, performance status testing; TIS, Taipei Integrated System; TNM, tumour node metastases; Vasc. invas., vascular invasion.
6 | AFP AS A TUMOUR ANTIGEN IN HCC

The relevance of the tumour microenvironment, and particularly the infiltrating immune cells, in HCC has been widely recognized. Recently, it has been shown that the immune contexture determines survival of HCC patients and that approximately 25% of HCCs belong to an immune-specific class defined by high expression levels of inflammatory response markers such as CD274 (programmed cell death ligand 1 [PD-L1]) and programmed cell death 1 (PD-1), among others. Immune checkpoint inhibitors, nivolumab and pembrolizumab, have shown efficacy with improved durable responses in nonrandomized, open-label, phase 2 trials. These findings suggest that HCC may be responsive to immunotherapy, particularly in at least a subgroup of patients. However, pembrolizumab did not meet the co-primary endpoints of significantly improved OS and PFS in a randomized phase 3 trial that included unselected patients with HCC who failed prior systemic therapy. How best to select patients and any impact of treatment sequence therefore remain important questions for characterizing the role of immunotherapy in HCC.

The effect of immunotherapy relies on the recognition of antigens expressed on cancer cells by the patient’s immune system, which subsequently attacks and eliminates the malignant cells. It has long been proposed that AFP as an oncofetal antigen can become a target for immunotherapy because it features potentially immunogenic epitopes and is not expressed in healthy individuals after birth. Naturally, the immune system is tolerant against AFP being a self-protein, and only low immunity is mounted against the protein in HCC patients despite high plasma levels. To overcome this tolerance, several AFP-based immune interventions have been tested in the past, which have, however, been mainly limited to animal models. Further studies are needed to demonstrate a benefit of AFP-based immunotherapies in HCC patients. In addition to the previously mentioned clinical trial (NCT03349255), another similar clinical trial in progress (NCT03132792) includes patients with HCC who have progressed on or were intolerant to prior therapy and have either tumour AFP levels ≥400 ng/mL or AFP expression of ≥1+ in ≥20% of tumour cells by immunohistochemistry, and noncancerous liver tissue with ≤5% cells that stain positive by immunohistochemistry for AFP. Included patients will be treated with autologous genetically modified AFP T cells that will specifically target the patient’s own AFP-expressing HCC tumour cells.

7 | CONCLUSIONS AND FUTURE DIRECTIONS

Hepatocellular carcinoma is a complex disease with multiple pathogenetic mechanisms caused by a variety of risk factors, making it difficult to characterize HCC with a single biomarker. Since its discovery more than 60 years ago, the use of AFP in clinical practice has evolved, and the knowledge of its role in HCC has expanded. Although AFP’s performance as a screening, diagnostic and prognostic marker for HCC is not ideal, it is the most frequently used biomarker in the management of HCC (summarized in Figure 4). Despite its considerable age, there are still open questions regarding the utility of AFP in the context of HCC that should be addressed: What are the optimal AFP cut-off values for HCC surveillance, diagnosis and prognosis? What combinations of AFP with other biomarkers (such as AFP-L3 or DCP) can significantly improve its performance in the various HCC settings? Does AFP have value in monitoring the response to treatment with more recent agents such as regorafenib or nivolumab? What functional role, if any, does it play in tumour development?

In the liver transplantation setting, AFP is in a strong position to be included in composite criteria, which consider surrogates of tumour biology, in addition to the conventional morphological factors such as tumour size and number of nodules. Here, the dimension of time may be taken into consideration to increase the value of AFP while patients are on a waiting list. Such composite criteria need to be investigated thoroughly, validated prospectively and auditable on demand.

Since the use of different cut-off values in past studies has prevented the use of widely accepted AFP thresholds in the clinic, it seems advisable for scientists to apply values that are common practice (eg 20 ng/mL for HCC screening and diagnosis) or have accumulated some evidence (eg 200 and/or 400 ng/mL for treatment stratification and HCC prognosis). In addition, serial measurements

| Surveillance | Diagnosis | Prognosis / monitoring of treatment response |
|--------------|-----------|---------------------------------------------|
| ✓            | ✓         | Hepatic resection                            |
| ✓            | ✓         | Liver transplantation                         |
| ✓            | ✓         | LRT                                          |
| ✓            | ✓         | Sorafenib                                    |
| ✓            | ✓         | Ramucirumab                                   |
| ✓            | ✓         | Lenvatinib                                    |
| ?            | ?         | Other TKIs                                    |
| ?            | ?         | Checkpoint Inhibitors                         |

FIGURE 4  The role of alpha-fetoprotein in the management of hepatocellular carcinoma
of AFP over time characterize the dynamic development of this marker, which may add to clinical judgement. In other areas, the future of AFP depends on broader developments, such as whether patient stratification according to molecular subclasses will mature into clinical practice or whether donor liver allocation algorithms will adopt one of the recently proposed criteria.

Like all great masters, AFP is challenged by the next generation. While AFP may be in its prime, its end is not in sight and it may remain HCC’s most important biomarker for years to come.

ACKNOWLEDGEMENTS

We thank Dr Carolin Czauderna (University Medical Center Mainz) for the contribution of Table 2 to this article. Eli Lilly and Company contracted with Syneos Health for writing support from Andrea Humphries, PhD, and editing support from Teri Tucker and Antonia Baldo.

CONFLICT OF INTEREST

PG was on advisory boards and received lecture fees from Bayer, BMS, MSD, Merck, SirteX, AstraZeneca, Sillajen, Eli Lilly and Company, Ipsen, Roche and Novartis. MK reports fees for advisory consulting from Bayer, Eisai, MSD and Ono; also grant money from Eisai, Diichi Sankyo, Medico’s Hirata, Otsuka, Taiho, Astellas Pharma, Chugai, Bristol-Myers Squibb, EA Pharma, Takeda and Gilead. JL received grants and personal fees from Bayer, Eisai Inc, Bristol Myers Squibb, Ipsen, Blueprint and Incyte, as well as personal fees from Eli Lilly and Company, Celsion Corporation, Exelixis, Merck, Clycostest, Navigant, Leerink Swann LLC, Midatech LTD, Fortress Biotech INC, Spring Bank Pharmaceuticals, and Nucleix, outside the submitted work. WS reports employment and stock ownership in Eli Lilly and Company and received research funding from Merck, Novartis, Bristol-Myers Squibb, Bayer and Eli Lilly and Company. SC has nothing to disclose.

ORCID

Peter R. Galle https://orcid.org/0000-0001-8294-0992
Friedrich Foerster https://orcid.org/0000-0002-3234-8891

REFERENCES

1. Forner A, Reig M, Bruix J. Hepatocellular carcinoma. Lancet. 2018;391:1301-1314.
2. Kim DY, Han KH. Epidemiology and surveillance of hepatocellular carcinoma. Liver Cancer. 2012;1:1-2.
3. Nault JC, Galle PR, Marquardt JU. The role of molecular enrichment on future therapies in hepatocellular carcinoma. J Hepatol. 2018;69:237-247.
4. AlSaloom AA. An update of biochemical markers of hepatocellular carcinoma. Int J Health Sci (Qassim). 2016;10:121-136.
5. Llovet JM, Ricci S, Mazzaferro V, et al. Sorafenib in advanced hepatocellular carcinoma. N Engl J Med. 2008;359:378-390.
6. Morse MA, Sun W, Kim R, et al. The role of angiogenesis in hepatocellular carcinoma. Clin Can Res. 2019;25:912-920.
7. Finn RS, Merle P, Granito A, et al. Outcomes of sequential treatment with sorafenib followed by regorafenib for HCC: Additional analyses from the phase III RESOURCE trial. J Hepatol. 2018;69:353-358.
8. Alsina A, Kudo M, Vogel A, et al. Subsequent anticancer medication following first-line lenvatinib: A posthoc responder analysis from the phase 3 REFLECT study in unresectable hepatocellular carcinoma [abstract]. J Clin Oncol. 2019;37(suppl 4):371.
9. Galle PR, Forner A, Llovet JM, et al. EASL clinical practice guidelines: management of hepatocellular carcinoma. J Hepatol. 2018;69:182-236.
10. Sturgeon CM, Duffy MJ, Hofmann BR, et al. National Academy of Clinical Biochemistry Laboratory Medicine Practice Guidelines for the use of tumor markers in liver, bladder, cervical, and gastric cancers. Clin Chem. 2010;56:e1-e48.
11. Marrero JA, Fung Z, Wang Y, et al. Alpha-fetoprotein, des-gamma carboxyprothrombin, and lectin-bound alpha-fetoprotein in early hepatocellular carcinoma. Gastroenterology. 2009;137:110-118.
12. Tanaka Y, Tateishi R, Koike K. Proteoglycans are attractive biomarkers and therapeutic targets in hepatocellular carcinoma. Int J Mol Sci. 2018;19:3070.
13. Marisi G, Cucchetti A, Ulivi P, et al. Ten years of sorafenib in hepatocellular carcinoma: are there any predictive and/or prognostic markers? World J Gastroenterol. 2018;24:4152-4163.
14. Bruix J, Cheng AL, Meinhardt G, Nakajima K, De SY, Llovet J. Prognostic factors and predictors of sorafenib benefit in patients with hepatocellular carcinoma: analysis of two phase III studies. J Hepatol. 2017;67:999-1008.
15. Horwitz E, Stein I, Andreozzi M, et al. Human and mouse VEGFA-amplified hepatocellular carcinomas are highly sensitive to sorafenib treatment. Cancer Discov. 2014;4:730-743.
16. Arao T, Ueshima K, Matsumoto K, et al. FGFR3/FGF4 amplification and multiple lung metastases in responders to sorafenib in hepatocellular carcinoma. Hepatology. 2013;57:1407-1415.
17. Llovet JM, Pena CE, Lathia CD, Shan M, Meinhardt G, Bruix J. Plasma biomarkers as predictors of outcome in patients with advanced hepatocellular carcinoma. Clin Cancer Res. 2012;18:2290-2300.
18. He L, Zhou X, Qu C, Tang Y, Zhang Q, Hong J. Serglycin (SRGN) overexpression predicts poor prognosis in hepatocellular carcinoma patients. Med Oncol. 2013;30:707.
19. Zhou L, Zhu Y. The EpCAM overexpression is associated with clinicopathological significance and prognosis in hepatocellular carcinoma patients: A systematic review and meta-analysis. Int J Surg. 2018;56:274-280.
20. Teufel M, Seidel H, Köchert K, et al. Biomarkers associated with response to regorafenib in patients with hepatocellular carcinoma. Gastroenterology. 2019;156:1731-1741.
21. Rimassa L, Assenat E, Peck-Radosavljevic M, et al. Tivantinib for second-line treatment of MET-high, advanced hepatocellular carcinoma (METIV-HCC): A final analysis of a phase 3, randomised, placebo-controlled study. Lancet Oncol. 2018;19:682-693.
22. Kudo M, Finn RS, Qin S, et al. Lenvatinib versus sorafenib in first-line treatment of patients with unresectable hepatocellular carcinoma: A randomised phase 3 non-inferiority trial. Lancet. 2018;391:1163-1173.
23. Finn RS, Kudo M, Cheng A-L, et al. Final analysis of serum biomarkers in patients (pts) from the phase III study of lenvatinib (LEN) vs sorafenib (SOR) in unresectable hepatocellular carcinoma (uHCC) [REFLECT] [abstract]. Ann Oncol. 2018;29:59PD.
24. Bergstrand CG, Czar B. Demonstration of a new protein fraction in serum from the human fetus. Scand J Clin Lab Invest. 1956;8:174.

25. Obiekwe BC, Malek N, Kitau MJ, Chard T. Maternal and fetal alpha-fetoprotein (AFP) levels at term. Relation to sex, weight and gestation of the infant. Acta Obstet Gynecol Scand. 1985;64:251-253.

26. Waller DK, Lustig LS, Cunningham GC, Feuchtbaum LB, Hook EB. The association between maternal serum alpha-fetoprotein and preterm birth, small for gestational age infants, preeclampsia, and placental complications. Obstet Gynecol. 1996;88:816-822.

27. Bader D, Riskin A, Vafsi O, et al. Alpha-fetoprotein in the early neonatal period—a large study and review of the literature. Clin Chim Acta. 2004;349:15-23.

28. Song YH, Naumova AK, Liebhaber SA, Cooke NE. Physical and meiotic mapping of the region of human chromosome 4q11-q13 encompassing the vitamin D binding protein DBP/Gc-globulin and albumin multigene cluster. Genome Res. 1999;9:581-587.

29. Lazarevich NL. Molecular mechanisms of alpha-fetoprotein gene expression. Biochemistry (Moscow). 2000;65:117-133.

30. Camper SA, Tilghman SM. Postnatal repression of the alpha-fetoprotein gene is enhancer independent. Genes Dev. 1989;3:537-546.

31. Terentiev AA, Moldogazieva NT. Alpha-fetoprotein: A renaissance. Tumour Biol. 2013;34:2075-2091.

32. Lamerz R. Are biomarkers still helpful in hepatocellular carcinoma? Digestion. 2013;87:118-120.

33. Zhao YJ, Ju Q, Li GC. Tumor markers for hepatocellular carcinoma. Mol Clin Oncol. 2013;1:593-598.

34. Kajiyama Y, Tian J, Locker J. Characterization of distant enhancers and promoters in the albumin-alpha-fetoprotein locus during active and silenced expression. J Biol Chem. 2006;281:30122-30131.

35. Shen H, Luan F, Liu H, et al. ZHK2 is a repressor of alpha-fetoprotein expression in human hepatoma cell lines. J Cell Mol Med. 2008;12:2772-2780.

36. Peterson ML, Ma C, Spear BT. Zhh2 and Zbtb20: novel regulators of postnatal alpha-fetoprotein repression and their potential role in gene reactivation during liver cancer. Semin Cancer Biol. 2011;21:21-27.

37. Kojima K, Takata A, Vadnais C, et al. MicroRNA122 is a key regulator of alpha-fetoprotein expression and influences the aggressiveness of hepatocellular carcinoma. Nat Commun. 2011;2:338.

38. Marchio A, Bertani S, Rojas Rojas T, et al. A peculiar mutation spectrum emerging from young Peruvian patients with hepatocellular carcinoma. PLoS ONE. 2014;9:e114912.

39. Sakata N, Kaneko S, Ikone S, et al. TGF-beta signaling cooperates with AT motif-binding factor-1 for repression of the alpha -fetoprotein promoter. J Signal Transduc. 2014;2014:970346.

40. Huang J, Qiu M, Wan L, et al. TGF-beta1 promotes hepatocellular carcinoma invasion and metastasis via ERK pathway-mediated FGFR4 expression. Cell Physiol Biochem. 2018;45:1690-1699.

41. Laderoute MP, Pilarski LM. The inhibition of apoptosis by alpha-fetoprotein (AFP) and the role of AFP receptors in anti-cellular senescence. Anticancer Res. 1994;14:2429-2438.

42. Semenkova LN, Dudich EI, Dudich IV, Shingarova LN, Korobko VG. Alpha-fetoprotein as a TNF resistance factor for the human hepatoma cell line HepG2. Tumour Biol. 1997;18:30-40.

43. Li MS, Li PF, He SP, Du GG, Li G. The promoting molecular mechanism of alpha-fetoprotein on the growth of human hepatoma Bel7402 cell line. World J Gastroenterol. 2002;8:469-475.

44. Dudich E, Semenkova L, Dudich I, Denesyuk A, Tatulov E, Korpela T. Alpha-fetoprotein antagonizes X-linked inhibitor of apoptosis protein anticaspase activity and disrupts XIAP-caspase interaction. FEBS J. 2006;273:3837-3849.

45. Lin BO, Zhu M, Wang W, et al. Structural basis for alpha fetoprotein-mediated inhibition of caspase-3 activity in hepatocellular carcinoma cells. Int J Cancer. 2017;141:1413-1421.

46. Gotsman I, Israeli D, Alper R, Rabbani E, Engelhardt D, Ilan Y. Induction of immune tolerance toward tumor-associated-antigens enables growth of human hepatoma in mice. Int J Cancer. 2002;97:52-57.

47. Toder V, Blank M, Gleicher N, Nebel L. Immunoregulatory mechanisms in pregnancy. II. Further characterization of suppressor lymphocytes induced by alpha-fetoprotein in lymphoid cell cultures. J Clin Lab Immunol. 1983;11:149-154.

48. Wang XW, Xu B. Stimulation of tumor-cell growth by alpha-fetoprotein. Int J Cancer. 1998;75:596-599.

49. Wang XW, Xie H. Alpha-fetoprotein enhances the proliferation of human hepatoma cells in vitro. Life Sci. 1999;64:17-23.

50. Goossens N, Sun X, Hoshida Y. Molecular classification of hepatocellular carcinoma: potential therapeutic implications. Hepat Oncol. 2015;2:371-379.

51. Boyault S, Rickman DS, de Reyniès A, et al. Transcriptome classification of HCC is related to gene alterations and to new therapeutic targets. Hepatology. 2007;45:42-52.

52. Hoshida Y, Nijman SM, Kobayashi M, et al. Integrative transcriptome analysis reveals common molecular subclasses of human hepatocarcinoma. Cancer Res. 2009;69:7385-7392.

53. Calderaro J, Couchy G, Imbeaud S, et al. Histological subtypes of hepatocarcinoma are related to gene mutations and molecular tumour classification. J Hepatol. 2017;67:727-738.

54. Llovet JM, Montal R, Sia D, Finn RS. Molecular therapies and precision medicine for hepatocellular carcinoma. Nat Rev Clin Oncol. 2018;15:599-616.

55. Yamashita T, Forgues M, Wang W, et al. EpCAM and alpha-fetoprotein expression defines novel prognostic subtypes of hepatocellular carcinoma. Cancer Res. 2008;68:1451-1461.

56. Zhu AX, Duda DG, Sahani DV, Jain RK. HCC and angiogenesis: Possible targets and future directions. Nat Rev Clin Oncol. 2011;8:292-301.

57. Connell LC, Harding JJ, Abou-Alfa GK. Advanced hepatocellular cancer: The current state of future research. Curr Treat Options Oncol. 2016;17:43.

58. Schoenleber SJ, Kurtz DM, Talwalkar JA, Roberts LR, Gores GJ. Prognostic role of vascular endothelial growth factor in hepatocellular carcinoma: Systematic review and meta-analysis. Br J Cancer. 2009;100:1385-1392.

59. Shan YF, Huang YL, Xie YK, et al. Angiogenesis and clinicopathologic characteristics in different hepatocellular carcinoma subtypes defined by EpCAM and alpha-fetoprotein expression status. Med Oncol. 2011;28:1012-1016.

60. Meng W, Li X, Bai Z, et al. Silencing alpha-fetoprotein inhibits VEGF and MMP-2/9 production in human hepatocellular carcinoma cell. PLoS ONE. 2014;9:e90660.

61. Tsukuma H, Hiyama T, Tanaka S, et al. Risk factors for hepatocellular carcinoma among patients with chronic liver disease. N Engl J Med. 1993;328:1771-1801.

62. Black AP, Mehta AS. The search for biomarkers of hepatocellular carcinoma and the impact on patient outcome. Curr Opin Pharmacol. 2018;41:74-78.

63. Di Bisceglie AM, Sterling RK, Chung RT, et al. Serum alpha-fetoprotein levels in patients with advanced hepatitis C: results from the HALT-C Trial. J Hepatol. 2005;43:434-441.

64. Sterling RK, Wright EC, Morgan TR, et al. Frequency of elevated hepatocellular carcinoma (HCC) biomarkers in patients with advanced hepatitis C. Am J Gastroenterol. 2012;107:64-74.

65. Collier J, Sherman M. Screening for hepatocellular carcinoma. Hepatology. 1998;27:273-278.

66. Trevisani F, D'Intino PE, Morselli-Labate AM, et al. Serum alpha-fetoprotein for diagnosis of hepatocellular carcinoma in patients with chronic liver disease: Influence of HBsAg and anti-HCV status. J Hepatol. 2001;34:570-575.
GALLE ET AL.

67. Colli A, Fraquelli M, Casazza G, et al. Accuracy of ultrasonography, spiral CT, magnetic resonance, and alpha-fetoprotein in diagnosing hepatocellular carcinoma: A systematic review. *Am J Gastroenterol*. 2006;101:513-523.

68. Colombo M. Screening for cancer in viral hepatitis. *Clin Liver Dis*. 2001;5:109-122.

69. Gupta S, Bent S, Kohlues J. Test characteristics of alpha-fetoprotein for detecting hepatocellular carcinoma in patients with hepatitis C. *A systematic review and critical analysis. Ann Intern Med*. 2003;139:46-50.

70. Lok AS, Sterling RK, Everhart JE, et al. Des-gamma-carboxy prothrombin and alpha-fetoprotein as biomarkers for the early detection of hepatocellular carcinoma. *Gastroenterology*. 2010;138:493-502.

71. Shim JJ, Kim JW, Lee CK, Jang JY, Kim BH. Oral antiviral therapy improves the diagnostic accuracy of alpha-fetoprotein levels in patients with chronic hepatitis B. *J Gastroenterol Hepatol*. 2014;29:1699-1705.

72. Wong G, Chan H, Tse Y-K, et al. On-treatment alpha-fetoprotein is a specific tumor marker for hepatocellular carcinoma in patients with chronic hepatitis B receiving entecavir. *Hepatology*. 2014;59:986-995.

73. Yang SW, Kim GH, Chung JW, et al. Prediction of risk for hepatocellular carcinoma by response of serum alpha-fetoprotein to entecavir therapy. *J Gastroenterol Hepatol*. 2015;30:1175-1182.

74. El-Serag HB, Kanwal F, Davila JA, Kramer J, Richardson P. A new laboratory-based algorithm to predict development of hepatocellular carcinoma in patients with hepatitis C and cirrhosis. *Gastroenterology*. 2014;146:1249-1255.

75. Tayob N, Stingo F, Do KA, Lok A, Feng Z. A Bayesian screening approach for hepatocellular carcinoma using multiple longitudinal biomarkers. *Biomетrics*. 2018;74:249-259.

76. Sherman M. Alphafetoprotein: An obituary. *J Hepatol*. 2001;34:603-605.

77. Zhang BH, Yang BH, Tang ZY. Randomized controlled trial of screening for hepatocellular carcinoma. *J Cancer Res Clin Oncol*. 2004;130:417-422.

78. Singal AG, Conjeevaram HS, Volk ML, et al. Effectiveness of hepatocellular carcinoma surveillance in patients with cirrhosis. *Cancer Epidemiol Biomarkers Prev*. 2012;21:793-799.

79. Omata M, Cheng A-L, Kokudo N, et al. Asia-Pacific clinical practice guidelines on the management of hepatocellular carcinoma: A 2017 update. *Hepatol Int*. 2017;11:317-370.

80. Heimbach JK, Kulik LM, Finn RS, et al. AASLD guidelines for the treatment of hepatocellular carcinoma. Hepatology. 2018;67:358-380.

81. Kudo M, Izumi N, Kokudo N, et al. Management of hepatocellular carcinoma in Japan: Consensus-Based Clinical Practice Guidelines proposed by the Japan Society of Hepatology (JSH) 2010 updated version. *Dig Dis*. 2011;29:339-364.

82. Kudo M, Matsui O, Izumi N, et al. JSH Consensus-Based Clinical Practice Guidelines for the Management of Hepatocellular Carcinoma: 2014 Update by the Liver Cancer Study Group of Japan. *Liver Cancer*. 2014;3:458-468.

83. Kitai S, Kudo M, Minami Y, et al. Validation of a new prognostic staging system for hepatocellular carcinoma: a comparison of the biomarker-combined Japan Integrated Staging Score, the conventional Japan Integrated Staging Score and the BALAD Score. *Oncology*. 2008;75(Suppl 1):83-90.

84. Tangkijvanich P, Anukulkarnkusol N, Suwangoop P, et al. Clinical characteristics and prognosis of hepatocellular carcinoma: analysis based on serum alpha-fetoprotein levels. *J Clin Gastroenterol*. 2000;31:302-308.

85. Ikai I, Arii S, Kojio M, et al. Reevaluation of prognostic factors for survival after liver resection in patients with hepatocellular carcinoma in a Japanese nationwide survey. *Cancer*. 2004;101:796-802.

86. Yang SH, Suh K-S, Lee HW, et al. A revised scoring system utilizing serum alphafetoprotein levels to expand candidates for living donor transplantation in hepatocellular carcinoma. *Surgery*. 2007;141:598-609.

87. Kwon C, Kim DJ, Han YS, et al. HCC in living donor liver transplantation: Can we expand the Milan criteria? *Dig Dis*. 2007;25:313-319.

88. Zheng S-S, Xu X, Wu J, et al. Liver transplantation for hepatocellular carcinoma: Hongzhou experiences. *Transplantation*. 2008;85:1726-1732.

89. Ravaoli M, Grazi GL, Piscaglia F, et al. Liver transplantation for hepatocellular carcinoma: Results of down-staging in patients initially outside the Milan selection criteria. *Am J Transplant*. 2008;8:2547-2557.

90. Toso C, Meeberg G, Hernandez-Alejandro R, et al. Total tumor volume and alpha-fetoprotein for selection of transplant candidates with hepatocellular carcinoma: A prospective validation. *Hepatology*. 2015;62:158-165.

91. Vibert E, Azoulay D, Hoti E, et al. Progression of alphafetoprotein before liver transplantation for hepatocellular carcinoma in cirrhotic patients: A critical factor. *Am J Transplant*. 2010;10:129-137.

92. Merani S, Majno P, Knetsman NM, et al. The impact of waiting list alpha-fetoprotein changes on the outcome of liver transplant for hepatocellular carcinoma. *J Hepatol*. 2011;55:814-819.

93. Duvoux C, Roudot-Thoraval F, Decaens T, et al. Liver transplantation for hepatocellular carcinoma: A model including α-fetoprotein improves the performance of Milan criteria. *Gastroenterology*. 2012;143:986-994.

94. Lai Q, Avolio AW, Manzia TM, et al. Combination of biological and morphological parameters for the selection of patients with hepatocellular carcinoma waiting for liver transplantation. *Clin Transplant*. 2012;26:E125-E131.

95. Vitale A, Volk ML, De Feo TM, et al; Liver Transplantation North Italy Transplant program (NITp) working group. A method for establishing allocation equity among patients with and without hepatocellular carcinoma on a common liver transplant waiting list. *J Hepatol*. 2014;60:290-297.

96. Lai Q, Nicolini D, Inostroza Nunez M, et al. A novel prognostic index in patients with hepatocellular cancer waiting for liver transplantation: Time-Radiological-response-Alpha-fetoprotein-Inflammation (TRAIN) Score. *Ann Surg*. 2016;264:787-796.

97. Sapisochin G, Goldaracena N, Laurence JM, et al. The extended Toronto criteria for liver transplantation in patients with hepatocellular carcinoma: a prospective validation study. *Hepatology*. 2016;64:2077-2088.

98. Halazun KJ, Najjar M, Abdelmessih RM, et al. Recurrence after liver transplantation for hepatocellular carcinoma: a new MORAL to the story. *Ann Surg*. 2017;265:557-564.

99. Lai Q, Vitale A, Iesari S, et al; European Hepatocellular Cancer Liver Transplant Study Group. Intention-to-treat survival benefit of liver transplantation in patients with hepatocellular cancer. *Hepatology*. 2017;66:1910-1919.

100. Maazaferrro V, Sposito C, Zhou J, et al. Metroticket 2.0 model for analysis of competing risks of death after liver transplantation for hepatocellular carcinoma. *Gastroenterology*. 2018;154:128-139.

101. Imamura H, Matsuyama Y, Tanaka E, et al. Risk factors contributing to early and late phase intrahepatic recurrence of hepatocellular carcinoma after hepatectomy. *J Hepatol*. 2003;38:200-207.

102. Han K, Tzimas GN, Barkun JS, et al. Preoperative alpha-fetoprotein slope is predictive of hepatocellular carcinoma recurrence after liver transplantation. *Can J Gastroenterol*. 2007;21:39-45.

103. Grätz M, Krason多地 M, Patkowski W, et al. Relevance of pre-transplant α-fetoprotein dynamics in liver transplantation for hepatocellular cancer. *Ann Transplant*. 2016;21:115-124.

104. Piñero F, Tisi Bañ M, de Ataide EC, et al; Latin American Liver Research, Education and Awareness Network (LALREAN). Liver
transplantation for hepatocellular carcinoma: evaluation of the alpha-fetoprotein model in a multicenter cohort from Latin America. Liver Int. 2016;36:1657-1667.

105. Notarpaolo A, Layese R, Magistri P, et al. Validation of the AFP model as a predictor of HCC recurrence in patients with viral hepatitis-related cirrhosis who had received a liver transplant for HCC. J Hepatol. 2017;66:552-559.

106. Mehta N, Dodge JL, Roberts JP, Yao FY. Validation of the prognostic power of the RETREAT score for hepatocellular carcinoma recurrence using the UNOS database. Am J Transplant. 2018;18:1206-1213.

107. Hong G, Suh K-S, Suh S-W, et al. Alpha-fetoprotein and (18)F-FDG positron emission tomography predict tumor recurrence better than Milan criteria in living donor liver transplantation. J Hepatol. 2016;64:852-859.

108. Gabr A, Abouchaleh N, Ali R, et al. Comparative study of post-transplant outcomes in hepatocellular carcinoma patients treated with chemoembolization or radioembolization. Eur J Radiol. 2017;93:100-106.

109. Cerban R, Ester C, Iacob S, et al. Predictive factors of tumor recurrence and survival in patients with hepatocellular carcinoma treated with transarterial chemoembolization. J Gastrointest Liver Dis. 2018;27:409-417.

110. Kudo M. Lenvatinib may drastically change the treatment landscape of hepatocellular carcinoma. Liver Cancer. 2018;7:1-19.

111. N’Kontchou G, Mahamoudi A, Aout M, et al. Radiofrequency ablation of hepatocellular carcinoma: long-term results and prognostic factors in 235 Western patients with cirrhosis. Hepatology. 2009;50:1475-1483.

112. Bruix J, Ortiz H, Meyer J, et al. New utility of an old marker: Serial alpha-fetoprotein measurements in prediction of tumor recurrence in patients undergoing systemic chemotherapy. J Clin Oncol. 2009;27:446-452.

113. Chan LT, Liu T-W, Chao Y, et al. Alpha-fetoprotein response predicts survival benefits of thalidomide in advanced hepatocellular carcinoma. Aliment Pharmacol Ther. 2005;22:217-226.

114. Liu L, Zhao Y, Jia J, et al. The prognostic value of alpha-fetoprotein response for advanced-stage hepatocellular carcinoma treated with sorafenib combined with transarterial chemoembolization. Sci Rep. 2016;6:19851.

115. Personeni N, Bozzarelli S, Pressiani T, et al. Usefulness of alpha-fetoprotein response in patients treated with sorafenib for advanced hepatocellular carcinoma. J Hepatol. 2012;57:101-107.

116. Shao YY, Lin ZZ, Hsu C, Shen YC, Hsu CH, Cheng AL. Early alpha-fetoprotein response predicts treatment efficacy of antiangiogenic systemic therapy in patients with advanced hepatocellular carcinoma. Cancer. 2010;116:4590-4596.

117. Mamdani H, Wu H, O’Neil BH, Sehdev A. Excellent response to Anti-PD-1 therapy in a patient with hepatocellular carcinoma: case report and review of literature. Discov Med. 2017;23:331-336.

118. Chevret S, Trinchet JC, Mathieu D, Rached AA, Beaumard M, Chastang C. A new prognostic classification for predicting survival in patients with hepatocellular carcinoma. Groupe d'Etude et de Traitement du Carcinome Hepatocellulaire. J Hepatol. 1999;31:133-141.

119. The Cancer of the Liver Italian Program (CLIP) Investigators. A new prognostic system for hepatocellular carcinoma: A retrospective study of 435 patients: the Cancer of the Liver Italian Program (CLIP) investigators. Hepatology. 1998;28:751-755.

120. Llovet JM, Bru C, Bruck J. Prognosis of hepatocellular carcinoma: The BCLC staging classification. Semin Liver Dis. 1999;19:329-338.

121. Leung T, Tang A, Zee B, et al. Construction of the Chinese University Prognostic Index for hepatocellular carcinoma and comparison with the TNM staging system, the Okuda staging system, and the Cancer of the Liver Italian Program staging system: A study based on 926 patients. Cancer. 2002;94:1760-1769.

122. Kudo M, Chung H, Osaki Y. Prognostic staging system for hepatocellular carcinoma (CLIP score): Its value and limitations, and a proposal for a new staging system, the Japanese Integrated Staging Score (JIS score). J Gastroenterol. 2003;38:207-215.

123. Kudo M, Chung H, Haji S, et al. Validation of a new prognostic staging system for hepatocellular carcinoma: The JIS score compared with the CLIP score. Hepatology. 2004;40:1396-1405.

124. Toyoda H, Kudama T, Osaki Y, et al. Staging hepatocellular carcinoma by a novel scoring system (BALAD score) based on serum markers. Clin Gastroenterol Hepatol. 2006;4:1528-1536.

125. Okuda K, Ohtsuji T, Obata H, et al. Natural history of hepatocellular carcinoma and prognosis in relation to treatment. Study of 850 patients. Cancer. 1985;56:918-928.

126. Tateshii R, Yoshida H, Shina S, et al. Proposal of a new prognostic model for hepatocellular carcinoma: an analysis of 403 patients. Gut. 2005;54:419-425.

127. Yau T, Yao TJ, Chan P, Ng K, Fan ST, Poon RT. A new prognostic score system in patients with advanced hepatocellular carcinoma not amenable to locoregional therapy: Implication for patient selection in systemic therapy trials. Cancer. 2008;113:2742-2751.

128. Zhang BH, Wang XH, Yue HY, Ling CQ. A new staging system is more discriminant than conventional staging systems for un-resectable hepatocellular carcinoma. J Cancer Res Clin Oncol. 2010;136:821-827.
139. Hsu C-Y, Huang Y-H, Hsia C-Y, et al. A new prognostic model for hepatocellular carcinoma based on total tumor volume: the Taipei Integrated Scoring System. J Hepatol. 2010;53:108-117.

140. Yau T, Tang VY, Yao TJ, Fan ST, Lo CM, Poon RT. Development of Hong Kong Liver Cancer staging system with treatment stratification for patients with hepatocellular carcinoma. Gastroenterology. 2014;146:1691-1700.

141. Berhane S, Toyoda H, Tada T, et al. Role of the GALAD and BALAD-2 serologic models in diagnosis of hepatocellular carcinoma and prediction of survival in patients. Clin Gastroenterol Hepatol. 2016;14:875-886.

142. Chan SL, Mo F, Johnson P, et al. Applicability of BALAD score in prognostication of hepatitis B-related hepatocellular carcinoma. J Gastroenterol Hepatol. 2015;30:1529-1535.

143. Zhu A, Kang Y, Yen C-J, et al. Ramucirumab after sorafenib in patients with advanced hepatocellular carcinoma and increased alpha-fetoprotein concentrations (REACH-2): a randomised, double-blind, placebo-controlled, phase 3 trial. Lancet Oncol. 2019;20:282-296.

144. De Stefano F, Chacon E, Turcios L, Marti F, Gedaly R. Novel biomarkers in hepatocellular carcinoma. Dig Liver Dis. 2018;50:1115-1123.

145. Foerster F, Hess M, Gerhold-Ay A, et al. The immune contexture of hepatocellular carcinoma predicts clinical outcome. Sci Rep. 2018;8:5351.

146. Sia D, Jiao Y, Martinez-Quetglas I, et al. Identification of an immune-specific class of hepatocellular carcinoma, based on molecular features. Gastroenterology. 2017;153:812-826.

147. El-Khoueiry AB, Sangro B, Yau T, et al. Nivolumab in patients with advanced hepatocellular carcinoma (CheckMate 040): an open-label, non-comparative, phase 1/2 dose escalation and expansion trial. Lancet. 2017;389:2492-2502.

148. Zhu AX, Finn RS, Edeline J, et al. Pembrolizumab in patients with advanced hepatocellular carcinoma previously treated with sorafenib (KEYNOTE-224): A non-randomised, open-label phase 2 trial. Lancet Oncol. 2018;19:940-952.

149. Merck. Merck provides update on KEYNOTE-240, a phase 3 study of KEYTRUDA® (pembrolizumab) in previously treated patients with advanced hepatocellular carcinoma. 2019. Available from: https://investors.merck.com/news/press-release-details/2019/Merck-Provides-Update-on-KEYNOTE-240-a-Phase-3-Study-of-KEYTRUDA-pembrolizumab-in-Previously-Treated-Patients-with-Advanced-Hepatocellular-Carcinoma/default.aspx. Accessed May 13, 2019.

150. Wang X, Wang Q. Alpha-fetoprotein and hepatocellular carcinoma immunity. Can J Gastroenterol Hepatol. 2018;2018:1-8.

151. Butterfield LH, Ribas A, Dissette VB, et al. A phase I/II trial testing immunization of hepatocellular carcinoma patients with dendritic cells pulsed with four alpha-fetoprotein peptides. Clin Cancer Res. 2006;12:2817-2825.

152. Wang XP, Wang QX, Lin HP, Xu B, Zhao Q, Chen K. Recombinant heat shock protein 70 functional peptide and alpha-fetoprotein epitope peptide vaccine elicits specific anti-tumor immunity. Oncotarget. 2016;7:71274-71284.

153. Grimm CF, Ortmann D, Mohr L, et al. Mouse alpha-fetoprotein-specific DNA-based immunotherapy of hepatocellular carcinoma leads to tumor regression in mice. Gastroenterology. 2000;119:1104-1112.

How to cite this article: Galle PR, Foerster F, Kudo M, et al. Biology and significance of alpha-fetoprotein in hepatocellular carcinoma. Liver Int. 2019;39:2214–2229. https://doi.org/10.1111/liv.14223