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REVIEWS

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Biology and significance of alpha-fetoprotein in hepatocellular carcinoma

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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

Abbreviations: AFP, alpha-fetoprotein; AFP-L3, fucosylated fraction of AFP; ALCPS, advanced liver cancer prognostic system; ALP, alkaline phosphatase; BCLC, Barcelona Clinic Liver Cancer; bm-JIS, biomarker-combined Japan Integrated Staging; CI, confidence interval; CIS, China Integrated Score; CLIP, Cancer of the Liver Italian Program; CUPI, Chinese University Prognostic Index; CUX1, Cut homeobox 1; DCP, des-gamma-carboxy prothrombin; EpCAM, epithelial cell adhesion molecule; Extrah. Spread, extrahepatic spread; HCC, hepatocellular carcinoma; HKLC, Hong Kong Liver Cancer classification; HR, hazard ratio; JIS, Japan Integrated Staging; OS, overall survival; PFS, progression-free survival; PPV, positive predictive value; PST, performance status testing; RFA, radiofrequency ablation; TACE, transcatheter arterial chemoembolization; TIS, Taipei Integrated System; TNM, tumour node metastases; Vasc. invas., vascular invasion; VEGF, vascular endothelial growth factor; ZBTB20, zinc-fingers and BTB domain containing 20; ZHX2, zinc-fingers and homeoboxes 2.

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.[2]

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.[13] 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.[14-17] 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.[12,18,19] 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.[20] 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.[21] 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.[22] 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.[23] 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.[23]

Despite the identification of candidate biomarkers, AFP is still the most widely accepted and used serum marker in HCC.[10] The aim of this review is to understand the significance and clinical relevance of AFP as a biomarker and tumour antigen in HCC.

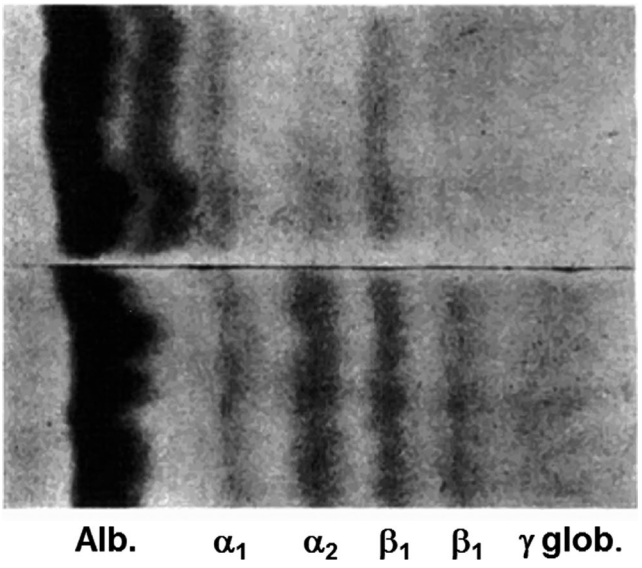

FIGURE 1 Fetal and maternal serum proteins fractions. Electrophoretic separation of serum proteins stained with Ponceau red from fetal (top) and maternal (bottom) blood samples. Fetal samples present an additional fraction between the albumin and α1 protein bands. Based on this position, the fraction was termed alpha-fetoprotein. From Bergstrand and Czar.[24] Copyright © Medisinsk Fysiologisk Forenings Forlag (MFFF), reprinted by permission of Taylor & Francis Ltd, http://www.tandfonline.com, on behalf of MFFF

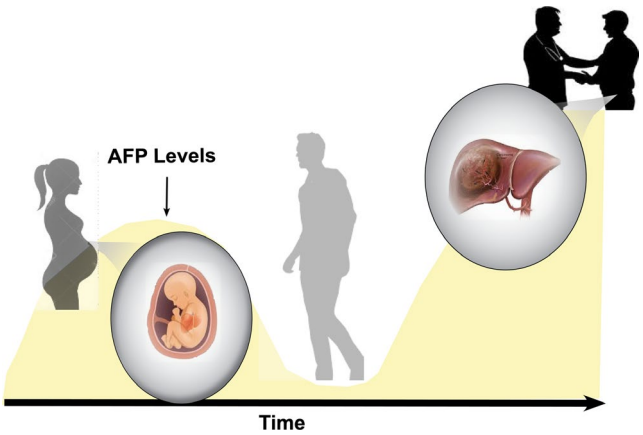

FIGURE 2 Relative levels of alpha-fetoprotein in the fetal liver, a healthy adult, or in a patient with hepatocellular carcinoma

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).[24] 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.[25] 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.[26] 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.[27]

The AFP gene is one of the four members of the albumin gene family localized in a tandem arrangement to form a multigene cluster,[28] 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

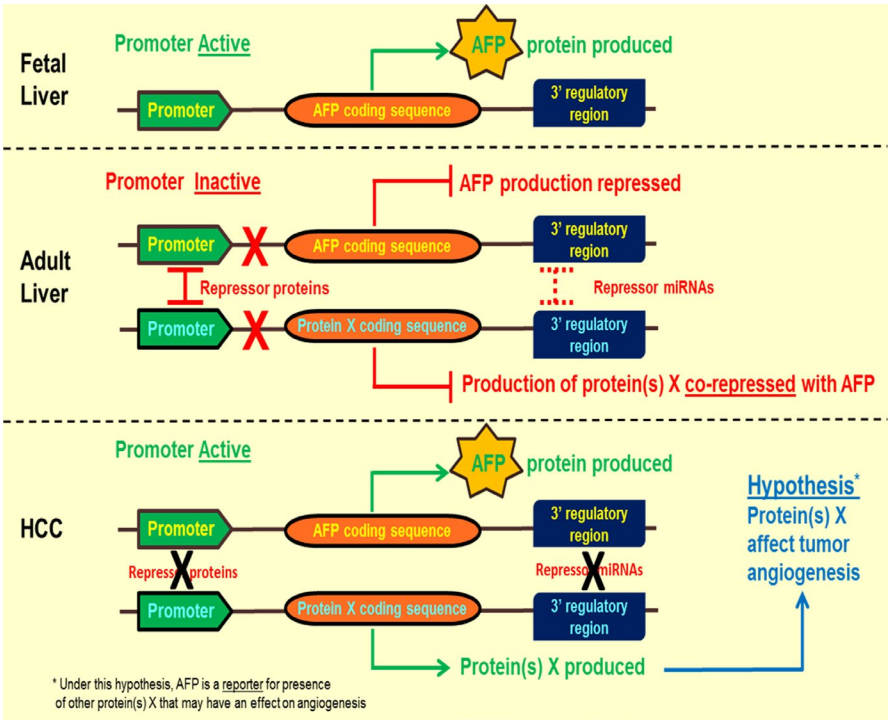

FIGURE 3 Concordant dysregulated expression of alpha-fetoprotein and other genes in the fetal liver, a healthy adult, or in a patient with hepatocellular carcinoma

by intracellular cAMP and protease A activity.[43] 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.[44] As a result, AFP may play a critical role in the inhibition of the apoptotic signal transduction mediated by caspase-3.[45]

Several studies also suggest that tumour growth results from AFP-mediated suppression of the antitumour immune response.[46] AFP was shown to interact with macrophages and to decrease their phagocytic activity and Ia 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[31]).

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.[31] 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_{14-20} might also cause a moderate stimulation of proliferation of lymphocytes and inhibition of proliferation of PHA-activated lymphocytes at concentrations of 10^7 to 10^9 M.[31] Since the heptapeptide AFP_{14-20} 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.[41,47-49]

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.[50] In addition, different transcriptomic subclasses have been described and linked to histological subtypes.[51,52] Through a global transcriptome analysis of 120 HCC tumours, Boyault et al[51] 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.[51] 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.[52] 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; $P < .001$).[52,53] Furthermore, AFP and EpCAM expression have been used to divide HCC patients into prognostic molecular subclasses.[3,54,55] 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.[56] The therapeutic effect of transarterial (chemo-) embolization and agents with antiangiogenic properties such as sorafenib support the vascular nature of these tumours.[57] High serum and tissue VEGF levels are associated with poor disease-free and OS in HCC.[58] In addition, VEGF has been reported as a prognostic biomarker in advanced HCC.[17] AFP and EpCAM, a hepatic stem cell expression marker, have also been implicated in the prognosis of HCC patients,[55] 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.[59] 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.[59] Likewise, silencing of AFP has been shown to inhibit VEGF production in HCC cells in vitro.[60] 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.[61,62] 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.[63-65] 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.[66]

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).[11,63,66-70] 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.[71,72] In turn, delayed AFP response to antiviral therapy may serve as an indicator of increased HCC risk.[73]

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.[67] 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.[74] 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.[75]

Given AFP'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.[76] 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.[77] 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%.[78] Consequently, practice guidelines currently recommend liver ultrasound with or without AFP levels as a screening method for patients at risk for HCC.[9,79,80] Guidelines from Japan recommend inclusion of AFP levels in surveillance programs.[81,82] The biomarker-combined Japan Integrated Staging (bm-JIS) score includes AFP, AFP-L3 and DCP specific for HCC to stratify patients and predict prognosis.[83] In the United States, guidelines suggest that serum AFP levels are optional for use in conjunction with ultrasound for screening at-risk populations.[80] 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.[9]

5.2 | AFP as a prognostic factor in HCC

Elevated AFP serum levels are associated with a poorer prognosis in HCC patients,[14,54] and serum AFP concentrations ≥400 ng/mL consistently denote poorer prognosis in different clinical settings.[84] 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).[85-106] Recently, the combination of pretransplant AFP (with a cut-off of 200 ng/mL) and ^{18}F-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.[107]

The rate of increase of AFP before liver transplantation (increase in AFP >50 ng/mL or >15 ng/mL per month)[91,102] has been shown to be a predictor of HCC recurrence and to be associated with worse survival after transplantation.[91] Interestingly, successful downstaging of AFP to 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 ($P \leq .001$), and an equivalent survival compared with patients whose AFP had always been ≤400 ng/mL.[92] 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.[108] Therefore, it has been suggested to include AFP levels in the selection of patients for inclusion on liver transplantation waiting lists.[97]

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.[5,22,109-116] 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%.[111,115] 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.[116]

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,[5] REFLECT,[22] RESORCE,[112] CELESTIAL[113,117,118] and REACH.[114] 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

TABLE 1 AFP in liver transplantation: prognosis and risk of recurrence

Study	N	Details regarding AFP	Survival rate/Recurrence risk[a]
AFP for selecting liver transplantation candidates			
Ikai et al 2004[85]	12 118	Preoperative serum AFP: <20 ng/mL 21-200 ng/mL 201-1000 ng/mL 1001-10 000 ng/mL ≥10 001 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 2007[86] (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 2007[87] (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 2008[88] (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%
Ravaioli et al 2008[89] (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%
Toso et al 2015[90] (TTV/AFP model)	233	AFP_L ≤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 2010[91]	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 2011[92]	6817	AFP_L >400 ng/mL downstaged to AFP ≤400 ng/mL AFP_L >400 ng/mL failed to reduce to ≤400 ng/mL AFP_L stable at ≤400 ng/mL	Intent-to-treat survival at 3 y: 81% 48% 74%
Duvoux et al 2012[93] (AFP model)	435	$\log_{10}$ AFP_L (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 2012[94] (AFP-TTD criteria)	158	Last AFP ≤400 ng/mL	Recurrence rate (median follow-up 43 mo): - In criteria: 4.9% - Outside criteria: 33.0%

(Continues)

TABLE 1 (Continued)

Study	N	Details regarding AFP	Survival rate/Recurrence risk[a]
Vitale et al 2014[95] (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 - 051 * logAFP + 4.59
Lai et al 2016[96] (TRAIN score)	179	AFP slope ≥15 ng/mL/month	ITT 5-year survival: - In criteria: 67.5% - Outside criteria: 23.5%
Sapisochin et al 2016[97] (extended Toronto criteria)	588	AFP_L ≥500 ng/mL <500 ng/mL	Actuarial patient survival: 60%, 43%, 37% 88%, 73%, 64%
Halazun et al 2017[98] (pre-MORAL score)	339	Maximum AFP from HCC diagnosis to LT >200 ng/mL	5-year RFS: - Low-risk (score 0-2): 98.6% - Medium-risk (score 3-6): 69.8% - High-risk (score 7-10): 55.8%
Lai et al 2017[99] (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
Mazzaferro et al 2018[100] (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) 5-year RFS: Within criteria of 89.6% vs beyond criteria of 46.8%
Risk of tumour recurrence			
Imamura et al 2003[101]	249	Preoperative AFP ≥32 ng/mL	Associated with recurrence within 2 y (HR 1.83, 95% CI: 1.25, 2.68)
Han et al 2007[102]	48	Preoperative AFP slope: >50 μg/L per month ≤50 μg/L per month	One-year RFS: 40% 90%
Grat et al 2016[103]	146	AFP persistently <100 ng/mL Initially high AFP dropped to <100 ng/mL AFP rising to >100 ng/mL AFP persistently at >100 ng/mL	5-year RFS: 97.3% 100% 75% 38.4%
Piñero et al 2016[104]	323	AFP_L: ≤100 ng/mL 101-1000 ng/mL >1000 ng/mL	5-year incidence of recurrence: 11.1% 19.7% 38.9%
Notarpaolo et al 2017[105]	574	Last AFP before LT: ≤100 ng/mL 100-1000 ng/mL >1000 ng/mL	5-year risk of recurrence: 13.0% 34.9% 75.0%
Mehta et al 2018[106] (RETREAT score)	3276	Preoperative AFP (ng/mL): - 0-20 - 21-99 - 100-999 ≥1000 ng/mL	3-year recurrence risk: - Score 0:1.6% - Score 1:5.0% - Score 2:5.6% - Score 3:8.4% - Score 4:20.3% - Score ≥5:29.0%

Abbreviations: AFP, alpha-fetoprotein; AFP_L, 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.

[a]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.

3 clinical trial design in advanced HCC.[112] 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.[119] 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.[120,121] 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.[122,123] Similar findings were subsequently observed in patients undergoing current standard systemic therapy including sorafenib, sorafenib in combination with TACE, cabozantinib monotherapy or ramucirumab monotherapy in the second-line setting when compared with placebo.[114,117,118,124,125] 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.[17,126] 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.[127] 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),[128] Cancer of the Liver Italian Program (CLIP) classification[129] and the BCLC staging system[130] are the European staging systems. The Chinese University Prognostic Index (CUPI score)[131] and the Japan Integrated Staging (JIS) system[132,133] are two Asian staging systems.

Two scores that include AFP as a parameter have been proposed.[83,134] 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 as well as AFP, has been shown to stratify HCC patients according to survival.[134] Biomarkers (AFP, 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.[83] The BALAD system has been validated in non-Japanese populations.[141,142] 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-blinded 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).[114] 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).[114] 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).[143] 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%).[143] This was the first positive 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

	Tumour markers			Lab chemistry				Clinical parameters				Tumour characteristics					
Study	AFP	AFP-L3	DCP	Albumin	Bili-rubin	Urea	ALP	Ascites	Child-Pugh	PST	Symptom-atic	Tumour size	Tumour morph.	Tumour count	Vasc. invas.	Extrah. spread	TNM
bm-JIS[83]	X	X	X						X								X
GRETCH[128]	X				X		X			X					X		
CLIP[129]	X								X				X		X		
BCLC[130]					X				X	X		X		X	X	X	
CUPI[131]	X				X		X	X			X						X
JIS[132]									X								X
BALAD[134]	X	X	X	X	X												
Okuda[135]				X	X			X				X					
Tokyo[136]				X	X							X		X			
ALCPS[137]	X					X	X	X	X		X	X			X	X	
CIS[138]	X								X								X
TIS[139]	X								X			X					
HKLC[140]									X	X		X			X	X	

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; CUPI, 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.

exclusively in patients with HCC tumours expressing AFP (>100 ng/mL) (Clinicaltrials.gov identifier NCT03349255).

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.[144] 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.[145,146] 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.[147,148] 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.[149] 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.[150] In addition, AFP promotes the proliferation of liver cancer, which makes it an even more worthy immunotherapeutic target.

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.[150] To overcome this tolerance, several AFP-based immune interventions have been tested in the past, which have, however, been mainly limited to animal models.[150-153] 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^{c332} 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 pathogenic 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

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.

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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, Clycotest, 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 is an employee of Eli Lilly and Company. PA reports employment with Eli Lilly and Company and pending patent WO2016/025464. AZ was a consultant for Eisai, Bristol-Myers Squibb, Merck, Novartis, Sanofi, Astrazeneca, Bayer, Eli Lilly and Company, and Exellxis. SQ, MS, FF and SC have nothing to disclose.

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