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Review Article

Incidence, Diagnosis, and Management of Hepatocellular Carcinoma: Current Perspectives and Future Direction

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Abstract

Hepatocellular carcinoma (HCC) is one of the most prevalent cancers worldwide, and due to substantial morbidity and mortality, has proven a significant global health-economic burden. Treatment options are broad and include surgical approaches (i.e., transplantation and resection), radiological (i.e., percutaneous ablation, and trans arterial approaches) and systemic therapies, though treatment response often remains poor. As such, clinical decision making requires a multidisciplinary approach to improve treatment strategy after consideration of the patient's tumor stage, liver function, and performance status. Current systemic cytotoxic therapies for non-surgical candidates have largely remained unchanged over the last decade. Systemic therapies have extended life expectancy by up to 3 months but not without potential notable adverse effects that often limit their use. However, even if patients have necessary access to best treatment, survival outcomes remain concerningly poor. Improved understanding of the pathogenic role of advanced liver fibrosis and wider cancer biology is spearheading the development of targeted immunogenic therapies that appear to offer real promise. Importantly, limiting progression to cirrhosis and early detection of HCC in at risk groups, alongside best use of currently accessible therapeutic options, remains key across global healthcare systems. The focus of this review is to critically assess all current published literature, encapsulating the prevalence, diagnosis, and management of HCC, whilst looking ahead to the potential future therapeutic directions in HCC management.

Keywords: Diagnosis; Imaging; Liver Cancer; Management; Surveillance; Systemic Therapy.

Abbreviations: AASLD: American Association for the Study of Liver Disease; AFP: Alpha-Fetoprotein; APASL: Asia-Pacific Association for the Study of the Liver; BCLC: Barcelona Clinic Liver Cancer; CT: Computed Tomography; EASL: European Association for the Study of the Liver; ECOG: The Eastern Cooperative Oncology Group; HCC: Hepatocellular Carcinoma; HBV: Hepatitis B Virus; HCV: Hepatitis C Virus; LT: Liver Transplantation; NAFLD: Non-Alcoholic Fatty Liver Disease; NASH: Non-Alcoholic Steatohepatitis; MELD: Model for End-Stage Liver Disease; MRI: Magnetic Resonance Imaging; TACE: Transarterial Chemoembolization; US: Ultrasound; WHO: World Health Organization

Introduction

Hepatocellular carcinoma (HCC) is one of the most lethal malignancies (~830000 deaths per year) and is an important medical problem globally. It is ranked as the fifth most common neoplasm and the second leading cause of cancer-related mortality, with a relative five-year survival rate of ~ 18% [1-4].

In Asian countries, the prevalence accounts for nearly 75-80 % of primary liver cancers [5]. In 2020, nearly one million people were diagnosed with liver cancer worldwide, the most dominant form of which reported was HCC [4]. Moreover, the World Health Organization (WHO) estimates that more than one million HCC patients will die in 2030 [6]. The burden of HCC varies according to demographic factors (age, gender, race/ethnicity), rarely occurs in people before age 40, increases more than 55 years, and reaches a peak at 70 years. Furthermore, the incidence rates among men are three times as high as the rates among women [7]. Consequently, due to its high prevalence, not many significant therapeutic options are available for advanced HCC. Therefore, improving the early detection and prognostication of HCC patients is imperative.

Incidence

Most cases of HCC arise from cirrhosis and additional comorbidities, and its incidence is expected to rise in the future [8, 9]. Hepatitis B virus (HBV) is a DNA virus that promotes mutation in liver cells by inducing necroinflammation and thus causes HCC and death worldwide (33%). By contrast, hepatitis C virus (HCV) is an RNA virus that does not integrate into the host genome and is thus unlikely to be the primary initiator of HCC. About 90% of HCV-associated liver cancer cases are heralded by cirrhosis, and the annual incidence rate ranges from 0.5% to 10% [5, 10, 11]. Moreover, chronic heavy alcohol consumption (>3 drinks/day) was associated with increased HCC risk (~16%); perhaps no association was noticed with lower levels of consumption (<3 drinks/day) [12]. Non-alcoholic fatty liver disease (NAFLD) and its more severe form, non-alcoholic steatohepatitis (NASH), is emerging as one of the leading HCC risk factors in developed regions [13, 14]. Recent evidence has emerged that obesity-mediated chronic inflammation was also associated with an increased risk of HCC [15, 16]. Interestingly, a diabetic individual with obesity shows an increased risk of liver cancer [1].

Prevention

Cirrhosis and other chronic liver diseases are susceptible to HCC, consequently, prevention may reduce the population at risk. Prevention of HCC can be achieved with universal vaccination against HBV infection [17]. The WHO recommends HBV vaccines for infants and high-risk groups. A previous study has shown in Taiwan that Nationwide HBV vaccination to infants resulted in a 36 % reduction in the incidence of HCC compared to unvaccinated cohorts [18]. Antiviral therapies effectively reduce HCC incidence in HBV-infected patients (HBsAg-positive), and eliminate HCV in viremia patients, however, does not eradicate the risk of HCC in viral hepatitis patients [7]. In this context, it has been shown that treatment with lamivudine (100mg/day) for 5 years to chronic HBV patients on the background of cirrhosis reduced the incidence of HCC risk compared to placebo [19]. Interferon therapy to HCV patients without cirrhosis had a sustained viral response and reduced HCC risk by about 75% compared to HCV patients with cirrhosis who do not have a sustained response to antiviral therapy [20, 21]. Furthermore, several studies conducted in Japan and southern Europe have shown that coffee drinking is associated with a reduced risk of HCC [22]. Albeit the mechanism for this protective effect remains poorly understood.

Surveillance/Screening

Surveillance and screening of HCC are tremendous approaches to detect the disease early and reduce mortality. So far, no high-quality randomized controlled trial has been available for the surveillance of HCC in cirrhotic patients [2]. However, several non-randomized studies have reported that HCC patients recruited into a surveillance programme had a chance of early diagnosis, more frequent curative therapy and better overall survival than unrecruited peers [23]. The imaging and serum α fetoprotein (AFP) measurement are the standard methods for surveillance of HCC. However, the use of AFP is no longer recommended due to its inadequate sensitivity (around 60%), specificity (80%) and predictive value for surveillance testing of HCC. Recently, many studies have identified a reliable biomarker in diagnosing AFP-negative HCC and thus ensuring the timely initiation of treatment (0599a pdf). Ultrasound (US) is the preferred imaging test for HCC surveillance and has a sensitivity ranging from 60-80% and a specificity of >90% [24, 25]. However, due to its operator dependency and unsatisfactory diagnostic accuracy, the use of US as a surveillance tool in clinical practice is limited [26].

Diagnosis

In general, an accurate and early diagnosis of HCC can improve the quality of life of HCC patients. Indeed, routinely followed clinical techniques such as imaging and histology could only detect late-stage diagnosis of HCC [27]. Globally, AFP is used as a conventional serum biomarker to detect HCC, albeit its levels remain normal in 30% of advanced HCC cases [28]. Moreover, elevated AFP is identified in benign liver diseases such as hepatitis and cirrhosis [29]. Consequently, the American association for the study of liver diseases (AASLD) practice guidelines no longer recommend AFP for the early detection of HCC [10]. Currently, many clinical and pre-clinical studies are focusing on identifying a new biomarker for diagnosing AFP-negative HCC, which may ensure the timely initiation of treatment. A large-scale multicenter study shows that serum DKK1, a Wnt/β-catenin signaling pathway inhibitor, could complement the diagnostic accuracy of AFP and improve the identification of patients with AFP-negative HCC and HCC from other chronic liver diseases [30]. In cirrhotic patients, HCC can be diagnosed based on validated imaging techniques or tissue biopsy. Multiphasic computed tomography (CT) or MRI is the commonly used imaging technique for HCC diagnosis if the diameter of the nodule is>1cm. However, these modalities represent a major clinical challenge if the nodule diameter is <1cm

[8, 2, 4]. Biopsy in advanced liver disease is safe and overcomes the limitations of non-invasive criteria since diagnostic certainty is needed to ensure the appropriate use of systemic therapy [31, 4]. Childs et al. also confirmed that biopsy in advanced liver disease predicts positivity in ~ 91% of HCC cases which proves about 9% of patients would receive inappropriate therapy in the absence of biopsy [31, 4]. However, the sensitivity of these noninvasive criteria is only 33% since a negative biopsy does not rule out HCC [32]. According to the AASLD and EASL guidelines, CD34, cytokeratin (CK) 7&19, GS, HSP70 and glypican 3 staining to improve diagnostic accuracy. EASL guidelines also supplement gene expression profiles of glypican 3 and survivin for HCC diagnosis. Arginase is a hepatocellular differentiation marker shown to differentiate less well-differentiated tumor from other liver tumors [4].

Clinical and biochemical markers

Vascular endothelial growth factor (VEGF) A and Angiotensin II are biomarkers of angiogenesis and have been associated with poor prognosis in HCC, albeit these markers failed to predict response to treatment [5, 11, 4]. Furthermore, biomarkers to predict treatment outcomes are lacking in HCC patients undergoing immunotherapy. Of note, an inflammatory marker such as CRAFITY (CRP and AFP in ImmunoTherapY) score is associated with survival and radiological response in HCC patients receiving anti-programmed death (ligand) (PD-L)1 immunotherapy but requires prospective validation [33]. The other inflammatory marker, neutrophil to lymphocyte ratio (NLR), is considered a prognostic predictor of HCC patients undergoing transarterial chemoembolization (TACE). Heat shock protein 90 alpha (Hsp90α), a molecular chaperone, is increased in HCC patients and positively correlated with tumor malignancy [34]. Compelling evidence indicates that micro RNAs (MiRs) are aberrantly expressed in HCC. In particular, MiRs are highly stable in circulation and can be used as a biomarker to test early-stage HCC [35]. In addition, osteopontin, glypican-3 and protein induced by vitamin K deficiency or antagonist-II (PIVKA-II), also known as Des-γ -carboxy-prothrombin (DCP), have been identified as serum biomarkers for early detection of HCC [36, 37]. Recently, we identified in HCC patients that tight junction protein zonula occludens (ZO) 1 blood levels were elevated and correlated well with serum hsCRP levels [38]. A recent study has shown that lens culinary agglutinin-reactive fraction of fetoprotein (AFP-L3), a subtype of AFP, is derived from cancerous hepatocytes used to diagnose early HCC [39]. However, AFP-L3 has not been recognized as a conventional diagnostic indicator of HCC. Saad et al. reported in HCV-related HCC patients (n=30) that serum levels of annexin A4 (ANXA4) might be a promising biomarker for the early diagnosis of HCC [40].

Prognosis assessment of HCC

Prognostic prediction is central in the management of HCC. For HCC patients with concurrent liver disease, the benefits of treating the tumor must be balanced against the potential harms of medical interventions already recommended to cirrhotic patients [2]. Thus, the complexity of managing HCC appeals for a multidisciplinary approach with expertise in hepatology, hepatobiliary surgery, radiology, pathology, oncology, and specialized nursing [10, 11, 2]. The prognostic assessment incorporated several measures, which include tumor burden (quantified based on the number and size), presence of macrovascular invasion, extrahepatic metastasis, degree of hepatic dysfunction (assessed by Child-Turcotte-Pugh score, MELD score, ascites, portal hypertension, albumin and bilirubin), and the Eastern cooperative oncology group (ECOG) performance status [11, 3]. Among the serological markers, elevated AFP level was correlated with poor prognosis and associated with the risk of tumor reoccurrence after surgical resection and liver transplant. Furthermore, a high DNA copy number of HBV was associated with poor prognosis and tumor reoccurrence [11, 3]. Several staging systems have been developed to assess the prognosis of HCC patients. The Barcelona-Clinic Liver Cancer (BCLC) staging system has been extensively validated and is the most widely applied staging system for HCC [5, 11, 2]. The other externally evaluated staging systems are the Cancer of the Liver Italian Program (CLIP), the French classification, Japan Integrated Staging (JIS), tumor, node, metastasis (TNM), the Hong-Kong Liver Cancer (HKLC) staging system, the Chinese University Prognostic Index (CUIP) and the Taipei integrated scoring system. According to the BCLC algorithm, HCC patients can be classified into five clinical stages, 0, A, B, C, and D, for a better treatment approach (Figure 1) [5, 8, 11, 2, 4].

BCLC 0: a very early stage of HCC with solitary nodule ≤ 2 cm without vascular invasion, Child-Pugh A, ECOG-PS 0.

BCLC A: early-stage HCC with solitary (>2 cm) or 2-3 nodules, all ≤ 3 cm, Child-Pugh A-B, ECOG-PS 0.

BCLC B: intermediate stage HCC with multinodular unresectable (>3 nodules or ≥2 nodules if any > 3cm), Child-Pugh A-B, ECOG-PS 0.

BCLC C: advanced HCC with symptomatic tumor, unresectable, segmental, or portal vein invasion, extrahepatic metastasis, Child-Pugh A-B, ECOG-PS 1-2.

BCLC D: end-stage liver function with non-transplantable HCC, Child-Pugh C, ECOG-PS 3-4.

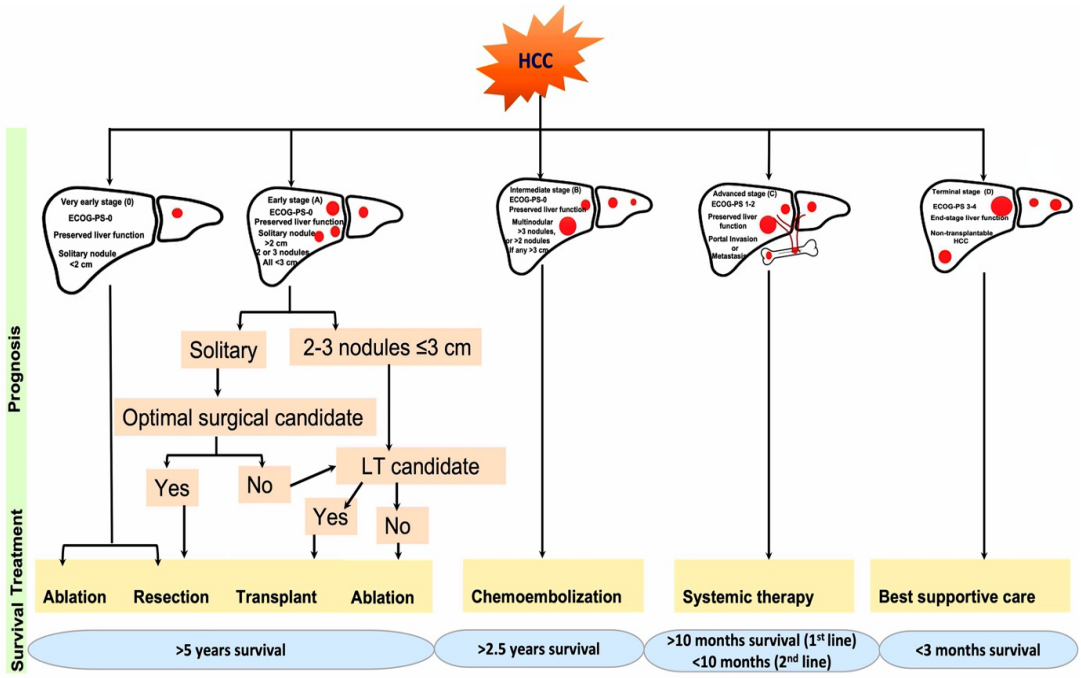

Figure 1: BCLC staging and treatment approach. According to the BCLC system, HCC can be categorized into five different stages of prognosis that are concurrent to first-line treatment recommendation. Indeed, to achieve the best clinical outcome, multidisciplinary team should meet up and carefully discuss the treatment plans. End-stage liver cirrhotic patients should be considered for LT due to precipitated liver function (high MELD and Child-Pugh class C or early stages with predictor of poor prognosis) [4,8,11]. Sorafenib followed by regorafenib as second-line therapy are effective in HCC patients. Lenvatinib has been shown to be non-inferior to sorafenib, however no second line therapy has been developed [4,8,11]. Moreover, Cabozantinib has been shown to be effective than placebo in 2nd and 3rd line with an improvement of OS [4,8,11]. Note: ECOG PS- Eastern cooperative oncology group performance status; HCC- hepatocellular carcinoma; LT- liver transplantation; OS- overall survival. 1st line treatment: Sorafenib and Levatinib; 2nd line treatment: Regorafenib, Cabozantinib and Ramucirumab.

Clinical Management

Several randomized controlled trials and cohort studies have revealed that numerous therapeutic approaches have exhibited survival benefits for HCC [5, 8, 11, 2, 4]. It arises from multiple etiologies, and almost 80-90% of HCC cases have underlying cirrhosis; therefore, the therapeutic option is limited due to the overall health status of the patients. The treatment protocol for HCC has been based on the BCLC algorithm, underlying disease severity, and expected benefits of the major intervention [5, 8, 11, 2, 4]. In principle, asymptomatic patients with low tumor burden and well-preserved liver function (BCLC stage 0/A) are assigned to be treated with local curative treatments (resection, ablation, or transplantation, depending upon the presence of portal hypertension, number of nodules, and liver function). Similarly, asymptomatic patients with multiple nodules and adequate liver function (BCLC stage B) are recommended to receive chemoembolization. In contrast, patients with portal thrombosis or extrahepatic metastasis (BCLC stage C) are allocated to treatment with first and second-line systemic chemotherapies [5, 8, 11, 2, 4]. HCC patients at their terminal stage (stage D) received the best supportive care and an estimated survival time of only three months [2].

Surgical resection

The ideal candidates for hepatic resection are patients without cirrhosis and an early-stage HCC (BCLC stage 0 or A), irrespective of tumor size and well-preserved liver function. In HCC patients with cirrhosis, hepatic resection is restricted with a single nodule (regardless of size), Child-Pugh A with total bilirubin <1 mg/dl, absence of clinically relevant portal hypertension (without ascites and varices), and ECOG score 0. For these patients, hepatic resection is associated with a 5-year survival of 70% with low postoperative mortality (<3%) [5, 10, 11, 2, 3]. However, many

of those 70% of patients have tumor recurrence at five years because the underlying chronic liver disease puts the patient at risk of developing new HCC [41]. Surprisingly, there is no data on adjuvant therapies to reduce recurrence in HCC patients [42]. In Asian countries and in USA, <5% of patients are candidates for surgical resection while in Asia, a greater number of young people with HBV-related HCC with minimal or no cirrhosis [7]. In the setting of HCV cirrhosis, about 75-80% of patients experience tumor recurrence following 5 years of resection [43].

Liver transplantation

Liver transplantation (LT) is recommended in HCC patients with a limited tumor burden (the Milan Criteria – single nodule ≤ 5 cm or 2-3 nodules ≤ 3cm without vascular invasion) and not on the hepatic resection list. LT has shown excellent survival outcomes with a 5-year survival of 70% and 10-year survival of 50% with only a 10-15% recurrence rate at 5 years [5, 10, 11]. Indeed, the long-term outcome of LT has shown to be superior to hepatic resection, which has a recurrence rate of 70% and a 10-year survival between 7-15% [5, 11, 3, 4]. Living donor LT is an alternative elective procedure that can eliminate dropout and enable LT in patients with HCC beyond the Milan criteria [43]. The calcineurin inhibitors (CNIs) such as cyclosporine and tacrolimus continue to remain potent immunosuppressants used in post LT [44] and have been shown to promote HCC progression through nonimmunologic mechanisms [43]. Moreover, the uncontrolled clinical study revealed that sirolimus, an alternative to tacrolimus, delay the appearance and retard the progression of recurrent HCC [45].

Non-surgical treatment

HCC patients with different tumor characteristics or with cirrhosis are not an ideal candidate for resection. Consequently, non-surgical procedures such as radiofrequency ablation (RFA), microwave ablations (MWA), percutaneous ethanol injection (PEI) and transarterial chemo embolization (TACE) have well proven anti-tumor effects. Image-guided ablation is restricted for small nodules < 2 cm as first-line treatment or as an alternative to hepatic resection for early-stage single nodules ≤ 4 cm or 2-3 nodules ≤ 3 cm [46, 43, 5, 11, 2-4]. For PEI, nodule diameter < 2 cm is still recommended. RFA is used as a first-line treatment for nodules < 2 cm or as an alternate surgery for early-stage single nodules ≤ 4 cm or 2-3 nodules ≤ 3 cm [46, 43, 5, 11, 2-4]. Moreover, the length of the hospital stay was shorter in the RFA group than in surgical resection. RFA has shown superior to percutaneous ethanol injection in improving OS. Indeed, RFA treatment has demonstrated median overall survival of 60 months and a 5-year recurrence of 50-70%. MWA has shown similar efficacy to RFA, however, MWA showed a higher complication rate in tumors > 3 cm [46, 43, 5, 11, 2-4]. Eventually, the success rate of both hepatic resection and PEI depends on careful follow-up and treatment of new tumors.

Globally, TACE has been recommended as first-line therapy for intermediate stage HCC patients (BCLC-B), particularly those with Child-Pugh A class cirrhosis who do not have extrahepatic metastasis or vascular invasion [7]. A systematic review of randomized trials for unresectable HCC showed survival benefits with TACE when compared to conservative treatment [47]. Furthermore, a systematic review of TACE showed an objective response of 52.5% and the mortality associated was below 1% [48]. Selective internal radiation therapy (SIRT) with yttrium-90 microspheres has recently been used as palliative treatment for BCLC stage B HCC patients. Indeed, no phase 3 trials compare yttrium-90 radiation therapy with TACE or other types of treatment with respect to survival [49]. Moreover, Vilgrain et al. showed in a phase 3 trial that SIRT to BCLC stage C HCC patients did not improve OS as compared with sorafenib and there was no improvement with a combination of SIRT with sorafenib when compared to sorafenib alone [50].

Systemic therapy

More than 70% of HCC patients are not amenable to treatment with LT or locoregional therapies and thus there is a great need for effective systemic therapies. Systemic therapy is the preferred treatment modality for advanced HCC patients (BCLC-C) and intermediate-stage HCC who do not qualify for local therapies. Over the past three decay, sorafenib was the first-line targeted therapy to show efficacy in advanced HCC patients. Sorafenib hepatocellular carcinoma assessment randomized protocol (SHARP) investigators study group showed median overall survival (OS) in the sorafenib arm was10.7-months compared to 7.9 months in the placebo group, representing a 31% decrease in the relative risk of death [51]. Of note, recently, Kelley et al. showed in a COSMIC-312 phase 3 trial, despite the lack of improvement in OS, cabozantinib plus atezolizumab significantly improved progression-free survival and showed increased disease control and lower primary progression compared with sorafenib [52]. In addition, Kudo et al. showed non-inferiority of lenvatinib (13.6 months) versus sorafenib (12.3 months) in terms of OS and improvement in progression-free survival, time to progression, and objective response rate thus, lenvatinib was superior to sorafenib [53]. A recent phase III trial (REFLECT study) confirms that FDA has approved Lenvatinib as first-line systemic therapy to advanced HCC [46, 43, 5, 11, 2-4]. Sunitinib is an oral multi-tyrosine kinase inhibitor (TKI) approved for treating other cancers but not recommended for HCC treatment due to safety issues and futility reasons [54]. Brivanib alaninate, an oral fibroblast growth factor (FGF)□FGF receptor (FGFR) and vascular endothelial growth factors (VEGFs) TKI used for advanced stage HCC with

the median OS was 10 months and 9.8 months in the first- and second-line treated groups, respectively with manageable adverse events [55].

In addition, three phase III trials showed negative results for primary endpoints when testing brivanib in the first line blinded to sorafenib, [56] in second line blinded to placebo [57] and in combination with chemoembolization [58]. The other TKI, linifanib, which targets VEGF and platelet-derived growth factor (PDGF), and ramucirumab, a monoclonal antibody against VEGFR2 [59], failed in phase III studies in first-line and second-line indications, respectively [60, 61]. Vatalanib, axitinib and cediranib are new anti-angiogenic agents involved in treating HCC, but the results are yet to come. Transforming growth factor-beta (TGFb), c-MET inhibitors, MEK (MAP2K1) inhibitors, and Janus kinase 2 (JAK2) inhibitors are also at the very early stage of investigation [62].

Regorafenib, an oral multi-kinase inhibitor used as second-line therapy, showed OS benefits over placebo (10.6 months vs 7.8 months) in HCC patients who tolerated and progressed on sorafenib in the phase III RESOURCE trial [63]. The FDA and the European medicines agency (EMA) have approved regorafenib to HCC patients who have already been treated with sorafenib. Similarly, cabozantinib (CELESTIAL study) showed superior to placebo in terms of OS (10.2 months vs 8.0 months) as second-line therapy [5, 11, 2-4]. Very recently, an updated result of the combination of atezolizumab + bevacizumab (IMbrave150 study) showed survival improvement over sorafenib as first-line therapy (19.2 months vs 13.4 months) [5, 11, 2-4]. However, these therapies are associated with weight loss, diarrhea, anorexia, asthenia, hand-foot reaction, hypertension, and proteinuria. Unfortunately, in the phase III trial, investigating a new agent or in combination with sorafenib as first-line or second-line therapy merely improved overall survival and failed to demonstrate a 5-year survival benefit [5, 11, 2-4]. Therefore, the discovery of new therapeutic agents is warranted, considering the survival benefit and adverse effects.

Immunotherapy

Immunotherapy has been proven effective and safe and improves survival rate and tolerable toxicity in HCC patients [64, 65]. The liver is a unique anatomical and immunological organ capable of producing antigen-specific tolerance and accepting LT. Therefore, the development of anti-tumor immunity against HCC is synergistically hindered by the tolerogenic properties of the liver and the immunosuppressive tumor microenvironment of HCC [66]. The US food and drug administration (FDA) has approved several immune checkpoint inhibitors (ICIs) for HCC and other cancers. These ICIs and inhibitory receptors include programmed cell death protein-1 (PD-1), or its ligand programmed cell death-ligand 1 (PD-L1) and cytotoxic T lymphocyte antigen 4 (CTLA-4), lymphocyte-activation gene 3 (LAG3), B and T lymphocyte attenuator (BTLA), T cell immunoglobulin and mucin domain containing-3 (TIM3) and T cell immunoreceptor with Ig and ITIM domains (TIGIT) [67]. The PD1 inhibitor nivolumab was used as the second-line therapy following sorafenib treatment to advance HCC patients. Moreover, many countries have recommended the PD-1 and PD-L1 inhibitors pembrolizumab and atezolizumab, respectively, as clinical treatment options for HCC. A clinical trial of the cytotoxic T-lymphocyte– associated protein 4 (CTLA-4) blockade tremelimumab showed a partial response rate of 17.6% in HCC patients with HCV [68]. Antiviral treatment shows improved liver function and histology and reduced HBV-DNA levels in HCC patients [69]. The primary nucleoside/nucleotide analogues (NAs), such as lamivudine treatment for HCC patients, reduced the incidence but did not eliminate early and mid-level HCC risk [70], however, entecavir therapy showed decreased HCC risk [71]. Of note, Papatheodoridis etal. showed in Caucasians with chronic HBV, following 5 years of entecavir or tenofovir treatment reduced the risk of HCC among persons with cirrhosis, but the overall risk was higher among cases without cirrhosis [70]. Moreover, numerous ongoing phase III trials exploring immune-based therapies may begin their role in the management of HCC.

Name of biomarkers	Potential Clinical application	Validation Method	References
Serum biomarker			
Glypican 3	Diagnostic and prognostic	Prospective, multi-centre phase II study	[72]
AFP-L3	Diagnostic marker – AFP negative cases	retrospective study	[73,74]
Osteopontin	Diagnostic and prognostic	Prospective, multi-centre study	[75]
PIVKAII	Diagnostic marker – AFP negative cases	large-scale, multicentre study	[76, 77]
Golgi protein-73	Diagnostic and prognostic	Prospective, single centre	[78,74, 79]
Annexin A4	Early diagnosis	Cross sectional – single centre	**[40]**
Heat shock protein 90alpha	Diagnostic marker	Cross sectional multicentre study	[80]
MicroRNA-4651	Diagnostic and prognostic - AFB1-positive cases	case-control study	[81]
miRNA classifier (Cmi)	a multicentre, retrospective, longitudinal case-control study		[82]
Metabolite biomarker panel	Diagnostic potential at-risk populations	A Large-scale, multicentre case-control study with AFP false-negative patients	[83]
Dermcidin	Diagnostic marker	case-control study	[84]
Tumor microenvironment			
a) Cellular components			
Tumor associated macrophages	Prognostic	Retrospective, single centre	[85]
Tumor-infiltrating lymphocytes	Prognostic	Meta-analysis	[86]
b) Non-cellular components			
Vascular endothelial growth factor	Prognosis -HBV Therapeutic target	Cross-sectional, single canter	[87]
Transforming growth factor-beta	Diagnosis and Prognosis – HBV	Cross-sectional, multi-centre	[88]
Cancer stem cell marker			
EpCAM	Therapeutic potential target	Retrospective, multi-centre	[89]
CD90	Prognostic. Associated to drug resistance	Retrospective, single centre	[89]
Note: Aflatoxin B1 (AFB1); miRNA classifier (Cmi) containing seven differentially expressed miRNAs (miR-29a, miR-29c, miR-133a, miR-143, miR-145, miR-192, and miR-505); Protein Induced by Vitamin K deficiency or antagonist-II (PIVKA-II), also known as Des-γ -carboxy-prothrombin (DCP); A serum metabolite biomarker - phenylalanyl-tryptophan and glycocholate.			

Table 1: Biomarkers and their clinical application in HCC.

Name of Drugs	Study outcome	Clinical trial design	References
Systemic therapyFirst-line therapy			
Sorafenib	Superior to overall survival	SHARP	NCT00105443 [51,90]
Lenvatinib	Lenvatinib was non-inferior to sorafenib in overall survival in untreated advanced HCC	Open-Label, Phase 3 REFLECT Trial	NCT01761266 [53, 91, 92]
Sunitinib	Negative outcome for HCC patients Pronounced toxicities	Randomized PRODIGE 16 trial, Intervention Model An Open Label Multi-Centre Phase 2 study	NCT01164202 [93] NCT00247676 [94]
Brivanib	Negative outcome for HCC patients	Multi-centre Phase III Study	NCT00858871 [58]
Erlotinib	Negative outcome for HCC patients	Open Label, Non-Randomized	NCT00287222 [95]
Linifanib	Negative outcome for HCC patients Ongoing	An Open-Label, Phase 2 Study An Open-label, Randomized Phase 3 Study An Open label Randomized Clinical Control Trial	NCT00517920 NCT01009593 [60] NCT05391867
1. Second-line therapy			
Cabozantinib	Improves overall survival and progression-free survival	Phase III CELESTIAL trial	NCT01908426 [96, 97]
Ramucirumab	Improves survival benefit	phase III REACH and REACH-2 randomized trials	NCT01140347 [98, 99] NCT02435433 [100]
Regorafenib	Improves overall survival in patients with HCC who had disease progression during first-line treatment with sorafenib	Multicentre Phase III RESORCE trial	NCT01774344 [63, 101, 102]
Nivolumab	ongoing	Phase Ib, Open label	NCT01658878
Pembrolizumab	ongoing	Phase II, Open label	NCT02702414
Everolimus	did not improve overall survival	the EVOLVE-1 randomized clinical trial	NCT01035229 [103]
Surgical treatments			
Liver transplantation	Increases survival	Non-population based, consecutive case series	[8]
Surgical resection	Increases survival	Non-population based, consecutive case series	[8]

Locoregional treatments			
Percutaneous treatment	Increases survival	Non-population based, consecutive case Series	[8]
Radiofrequency	Increases survival	Non-blinded, randomised controlled trial,meta-analysis	[8]
Chemoembolization	Increases survival	Non-blinded, randomised controlled trial, meta-analysis	[8]

Table 2: Molecular targeted therapies for advanced HCC.

Conclusion and Future Perspectives

HCC is a growing health problem, and globally we are expected to see over one million new cases each year by 2025. HCC is a complex disease predominantly seen on a background of advanced liver cirrhosis, a condition already associated with significant morbidity and mortality, from associated complications, with a yearly incidence of HCC development evident in around 1-5% of patients with cirrhosis compounding the problem. In at risk groups, early detection via a dedicated screening programme is pivotal and has a profound impact on outcomes. Moreover, in those later diagnosed with HCC, a multidisciplinary approach with the necessary full complement of best treatment options, whether surgical, radiological and/or oncological, ultimately provide best treatment outcomes. Over the past decade, with the introduction of global guidelines, HCC cancer networks and the introduction of systemic therapies like Sorafenib, the clinical management of HCC has evolved considerably, though ultimately any improvements in outcomes remained modest. The big challenge regarding advanced non-surgical approaches to HCC management is identifying novel combination regimens for greater and continued improvement in outcome in the front-line setting. Any new therapy has to be compared to Sorafenib, which represents the gold standard for systemic therapy in clinical trials and clinical care. However, there is still the possibility of seeing further improvements with Sorafenib as part of combination therapy and thus further phase III trials are urgently needed to evaluate sorafenib as adjuvant therapy after curative or locoregional therapies. Moreover, additional second-line therapies are required if sorafenib is unsuccessful in advanced stage HCC. Future trials involving effective systemic therapies, especially immunotherapies based on (i.e., checkpoint inhibitors) should continue to rise along with the pursuit of new biomarkers that enable personalized and cost-effective therapeutic stratification and advancement in managing all stages of HCC.

Declaration

We declare that we have no conflicts of interest. All the authors contributed equally to this review.

Consent for publication

All the authors provide consent for publishing the manuscript.

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References

1. Bosetti C, Turati F, La Vecchia C. Hepatocellular carcinoma epidemiology. Best Pract Res Clin Gastroenterol. 2014 Oct;28(5):753-70.
2. Villanueva A. Hepatocellular Carcinoma. N Engl J Med. 2019 Apr 11;380(15):1450-62.
3. Llovet JM, Kelley RK, Villanueva A, Singal AG, Pikarsky E, Roayaie S, et al. Hepatocellular carcinoma. Nat Rev Dis Primers. 2021 Jan 21;7(1):6.
4. Vogel A MT, Sapisochin G, Salem R, Saborowski, A. Hepatocellular carcinoma. The Lancet. 2022:1-18.
5. O'mata M CA, Kokudo N, Kudo M et al. Asia–Pacific clinical practice guidelines on the management of hepatocellular carcinoma: a 2017 update. Hepatol Int. 2017;11:317-70.
6. Organaization. WH. Cancer. 2017.
7. El-Serag HB. Hepatocellular carcinoma. N Engl J Med. 2011 Sep 22;365(12):1118-27.
8. Forner A, Reig M, Bruix J. Hepatocellular carcinoma. Lancet. 2018 Mar 31;391(10127):1301-14.
9. Vitale A, Trevisani F, Farinati F, Cillo U. Treatment of Hepatocellular Carcinoma in the Precision Medicine Era: From Treatment Stage Migration to Therapeutic Hierarchy. Hepatology. 2020 Dec;72(6):2206-18.
10. Heimbach JK, Kulik LM, Finn RS, Sirlin CB, Abecassis MM, Roberts LR, et al. AASLD guidelines for the treatment of hepatocellular carcinoma. Hepatology. 2018 Jan;67(1):358-80.
11. Liver EAftSot. EASL Clinical Practice Guidelines: Management of hepatocellular carcinoma. Journal of Hepatology. 2018;69:182-236.
12. Turati F, Galeone C, Rota M, Pelucchi C, Negri E, Bagnardi V, et al. Alcohol and liver cancer: a systematic review and meta-analysis of

prospective studies. Ann Oncol. 2014 Aug;25(8):1526-35.

13. Dyson J, Jaques B, Chattopadyhay D, Lochan R, Graham J, Das D, et al. Hepatocellular cancer: the impact of obesity, type 2 diabetes and a multidisciplinary team. J Hepatol. 2014 Jan;60(1):110-7.
14. Kanwal F, Kramer JR, Duan Z, Yu X, White D, El-Serag HB. Trends in the Burden of Nonalcoholic Fatty Liver Disease in a United States Cohort of Veterans. Clin Gastroenterol Hepatol. 2016 Feb;14(2):301-8 e1-2.
15. Therasse P, Arbuck SG, Eisenhauer EA, Wanders J, Kaplan RS, Rubinstein L, et al. New guidelines to evaluate the response to treatment in solid tumors. European Organization for Research and Treatment of Cancer, National Cancer Institute of the United States, National Cancer Institute of Canada. J Natl Cancer Inst. 2000 Feb 2;92(3):205-16.
16. Sun B, Karin M. Obesity, inflammation, and liver cancer. J Hepatol. 2012 Mar;56(3):704-13.
17. Chang MH, You SL, Chen CJ, Liu CJ, Lai MW, Wu TC, et al. Long-term Effects of Hepatitis B Immunization of Infants in Preventing Liver Cancer. Gastroenterology. 2016 Sep;151(3):472-80 e1.
18. Liao SH, Chen CL, Hsu CY, Chien KL, Kao JH, Chen PJ, et al. Long-term effectiveness of population-wide multifaceted interventions for hepatocellular carcinoma in Taiwan. J Hepatol. 2021 Jul;75(1):132-41.
19. Liaw YF, Sung JJ, Chow WC, Farrell G, Lee CZ, Yuen H, et al. Lamivudine for patients with chronic hepatitis B and advanced liver disease. N Engl J Med. 2004 Oct 7;351(15):1521-31.
20. Di Bisceglie AM, Shiffman ML, Everson GT, Lindsay KL, Everhart JE, Wright EC, et al. Prolonged therapy of advanced chronic hepatitis C with low-dose peginterferon. N Engl J Med. 2008 Dec 4;359(23):2429-41.
21. Singal AG, Volk ML, Jensen D, Di Bisceglie AM, Schoenfeld PS. A sustained viral response is associated with reduced liver-related morbidity and mortality in patients with hepatitis C virus. Clin Gastroenterol Hepatol. 2010 Mar;8(3):280-8, 88 e1.
22. Larsson SC, Wolk A. Coffee consumption and risk of liver cancer: a meta-analysis. Gastroenterology. 2007 May;132(5):1740-5.
23. Sherman M. Surveillance for hepatocellular carcinoma. Best Pract Res Clin Gastroenterol. 2014 Oct;28(5):783-93.
24. Bolondi L. Screening for hepatocellular carcinoma in cirrhosis. J Hepatol. 2003 Dec;39(6):1076-84.
25. Singal A, Volk ML, Waljee A, Salgia R, Higgins P, Rogers MA, et al. Meta-analysis: surveillance with ultrasound for early-stage hepatocellular carcinoma in patients with cirrhosis. Aliment Pharmacol Ther. 2009 Jul;30(1):37-47.
26. Singal AG, Nehra M, Adams-Huet B, Yopp AC, Tiro JA, Marrero JA, et al. Detection of hepatocellular carcinoma at advanced stages among patients in the HALT-C trial: where did surveillance fail? Am J Gastroenterol. 2013 Mar;108(3):425-32.
27. Luo P, Wu S, Yu Y, Ming X, Li S, Zuo X, et al. Current Status and Perspective Biomarkers in AFP Negative HCC: Towards Screening for and Diagnosing Hepatocellular Carcinoma at an Earlier Stage. Pathol Oncol Res. 2020 Apr;26(2):599-603.
28. Han LL, Lv Y, Guo H, Ruan ZP, Nan KJ. Implications of biomarkers in human hepatocellular carcinoma pathogenesis and therapy. World J Gastroenterol. 2014 Aug 14;20(30):10249-61.
29. Chen S, Chen H, Gao S, Qiu S, Zhou H, Yu M, et al. Differential expression of plasma microRNA-125b in hepatitis B virus-related liver diseases and diagnostic potential for hepatitis B virus-induced hepatocellular carcinoma. Hepatol Res. 2017 Mar;47(4):312-20.
30. Shen Q, Fan J, Yang XR, Tan Y, Zhao W, Xu Y, et al. Serum DKK1 as a protein biomarker for the diagnosis of hepatocellular carcinoma: a large-scale, multicentre study. Lancet Oncol. 2012 Aug;13(8):817-26.
31. Childs A, Zakeri N, Ma YT, O'Rourke J, Ross P, Hashem E, et al. Biopsy for advanced hepatocellular carcinoma: results of a multicentre UK audit. Br J Cancer. 2021 Nov;125(10):1350-55.
32. Forner A, Vilana R, Ayuso C, Bianchi L, Sole M, Ayuso JR, et al. Diagnosis of hepatic nodules 20 mm or smaller in cirrhosis: Prospective validation of the noninvasive diagnostic criteria for hepatocellular carcinoma. Hepatology. 2008 Jan;47(1):97-104.
33. Scheiner B, Pomej K, Kirstein MM, Hucke F, Finkelmeier F, Waidmann O, et al. Prognosis of patients with hepatocellular carcinoma treated with immunotherapy - development and validation of the CRAFITY score. J Hepatol. 2022 Feb;76(2):353-63.
34. Wang X, Song X, Zhuo W, Fu Y, Shi H, Liang Y, et al. The regulatory mechanism of Hsp90alpha secretion and its function in tumor malignancy. Proc Natl Acad Sci U S A. 2009 Dec 15;106(50):21288-93.
35. Lyra-Gonzalez I, Flores-Fong LE, Gonzalez-Garcia I, Medina-Preciado D, Armendariz-Borunda J. MicroRNAs dysregulation in hepatocellular carcinoma: Insights in genomic medicine. World J Hepatol. 2015 Jun 18;7(11):1530-40.
36. Bertino G, Ardiri A, Malaguarnera M, Malaguarnera G, Bertino N, Calvagno GS. Hepatocellualar carcinoma serum markers. Semin Oncol. 2012 Aug;39(4):410-33.
37. Debes JD RP, Prieto J, Arrese M, Mattos AZ, Boonstra A. Serum biomarkers for the prediction of hepatocellular carcinoma. Cancers. 2021;13:1681.
38. Ram AK, Pottakat B, Vairappan B. Increased systemic zonula occludens 1 associated with inflammation and independent biomarker in patients with hepatocellular carcinoma. BMC Cancer. 2018 May 18;18(1):572.
39. Zhou JM, Wang T, Zhang KH. AFP-L3 for the diagnosis of early hepatocellular carcinoma: A meta-analysis. Medicine (Baltimore). 2021 Oct 29;100(43):e27673.
40. Saad ZM, Fouad Y, Ali LH, Hassanin TM. Clinical Significance of Annexin A4 as a Biomarker in the Early Diagnosis of Hepatocellular Carcinoma. Asian Pac J Cancer Prev. 2020 Sep 1;21(9):2661-65.
41. Ishizawa T, Hasegawa K, Aoki T, Takahashi M, Inoue Y, Sano K, et al. Neither multiple tumors nor portal hypertension are surgical contraindications for hepatocellular carcinoma. Gastroenterology. 2008 Jun;134(7):1908-16.
42. Bruix J, Takayama T, Mazzaferro V, Chau GY, Yang J, Kudo M, et al. Adjuvant sorafenib for hepatocellular carcinoma after resection or ablation (STORM): a phase 3, randomised, double-blind, placebo-controlled trial. Lancet Oncol. 2015 Oct;16(13):1344-54.
43. Schwartz M, Roayaie S, Konstadoulakis M. Strategies for the management of hepatocellular carcinoma. Nat Clin Pract Oncol. 2007 Jul;4(7):424-32.
44. Saliba F, Duvoux C, Dharancy S, Dumortier J, Calmus Y, Gugenheim J, et al. Five-year outcomes in liver transplant patients receiving

everolimus with or without a calcineurin inhibitor: Results from the CERTITUDE study. Liver Int. 2022 Aug 13.

45. Kneteman NM, Oberholzer J, Al Saghier M, Meeberg GA, Blitz M, Ma MM, et al. Sirolimus-based immunosuppression for liver transplantation in the presence of extended criteria for hepatocellular carcinoma. Liver Transpl. 2004 Oct;10(10):1301-11.

46. Burrel M, Llovet JM, Ayuso C, Iglesias C, Sala M, Miquel R, et al. MRI angiography is superior to helical CT for detection of HCC prior to liver transplantation: an explant correlation. Hepatology. 2003 Oct;38(4):1034-42.

47. Llovet JM, Bruix J. Systematic review of randomized trials for unresectable hepatocellular carcinoma: Chemoembolization improves survival. Hepatology. 2003 Feb;37(2):429-42.

48. Lencioni R, de Baere T, Soulen MC, Rilling WS, Geschwind JF. Lipiodol transarterial chemoembolization for hepatocellular carcinoma: A systematic review of efficacy and safety data. Hepatology. 2016 Jul;64(1):106-16.

49. Salem R, Gordon AC, Mouli S, Hickey R, Kallini J, Gabr A, et al. Y90 Radioembolization Significantly Prolongs Time to Progression Compared With Chemoembolization in Patients With Hepatocellular Carcinoma. Gastroenterology. 2016 Dec;151(6):1155-63 e2.

50. Vilgrain V, Pereira H, Assenat E, Guiu B, Ilonca AD, Pageaux GP, et al. Efficacy and safety of selective internal radiotherapy with yttrium-90 resin microspheres compared with sorafenib in locally advanced and inoperable hepatocellular carcinoma (SARAH): an open-label randomised controlled phase 3 trial. Lancet Oncol. 2017 Dec;18(12):1624-36.

51. Llovet JM, Ricci S, Mazzaferro V, Hilgard P, Gane E, Blanc JF, et al. Sorafenib in advanced hepatocellular carcinoma. N Engl J Med. 2008 Jul 24;359(4):378-90.

52. Kelley RK, Rimassa L, Cheng AL, Kaseb A, Qin S, Zhu AX, et al. Cabozantinib plus atezolizumab versus sorafenib for advanced hepatocellular carcinoma (COSMIC-312): a multicentre, open-label, randomised, phase 3 trial. Lancet Oncol. 2022 Aug;23(8):995-1008.

53. Kudo M, Finn RS, Qin S, Han KH, Ikeda K, Piscaglia F, et al. Lenvatinib versus sorafenib in first-line treatment of patients with unresectable hepatocellular carcinoma: a randomised phase 3 non-inferiority trial. Lancet. 2018 Mar 24;391(10126):1163-73.

54. Cheng AL, Kang YK, Lin DY, Park JW, Kudo M, Qin S, et al. Sunitinib versus sorafenib in advanced hepatocellular cancer: results of a randomized phase III trial. J Clin Oncol. 2013 Nov 10;31(32):4067-75.

55. Park JH, McMillan DC, Powell AG, Richards CH, Horgan PG, Edwards J, et al. Evaluation of a tumor microenvironment-based prognostic score in primary operable colorectal cancer. Clin Cancer Res. 2015 Feb 15;21(4):882-8.

56. Johnson PJ, Qin S, Park JW, Poon RT, Raoul JL, Philip PA, et al. Brivanib versus sorafenib as first-line therapy in patients with unresectable, advanced hepatocellular carcinoma: results from the randomized phase III BRISK-FL study. J Clin Oncol. 2013 Oct 1;31(28):3517-24.

57. Llovet JM, Decaens T, Raoul JL, Boucher E, Kudo M, Chang C, et al. Brivanib in patients with advanced hepatocellular carcinoma who were intolerant to sorafenib or for whom sorafenib failed: results from the randomized phase III BRISK-PS study. J Clin Oncol. 2013 Oct 1;31(28):3509-16.

58. Kudo M, Han G, Finn RS, Poon RT, Blanc JF, Yan L, et al. Brivanib as adjuvant therapy to transarterial chemoembolization in patients with hepatocellular carcinoma: A randomized phase III trial. Hepatology. 2014 Nov;60(5):1697-707.

59. Spratlin JL, Cohen RB, Eadens M, Gore L, Camidge DR, Diab S, et al. Phase I pharmacologic and biologic study of ramucirumab (IMC-1121B), a fully human immunoglobulin G1 monoclonal antibody targeting the vascular endothelial growth factor receptor-2. J Clin Oncol. 2010 Feb 10;28(5):780-7.

60. Cainap C, Qin S, Huang WT, Chung IJ, Pan H, Cheng Y, et al. Linifanib versus Sorafenib in patients with advanced hepatocellular carcinoma: results of a randomized phase III trial. J Clin Oncol. 2015 Jan 10;33(2):172-9.

61. Zhu AX, Park JO, Ryoo BY, Yen CJ, Poon R, Pastorelli D, et al. Ramucirumab versus placebo as second-line treatment in patients with advanced hepatocellular carcinoma following first-line therapy with sorafenib (REACH): a randomised, double-blind, multicentre, phase 3 trial. Lancet Oncol. 2015 Jul;16(7):859-70.

62. Villanueva A, Llovet JM. Targeted therapies for hepatocellular carcinoma. Gastroenterology. 2011 May;140(5):1410-26.

63. Bruix J, Qin S, Merle P, Granito A, Huang YH, Bodoky G, et al. Regorafenib for patients with hepatocellular carcinoma who progressed on sorafenib treatment (RESORCE): a randomised, double-blind, placebo-controlled, phase 3 trial. Lancet. 2017 Jan 7;389(10064):56-66.

64. Schizas D, Charalampakis N, Kole C, Economopoulou P, Koustas E, Gkotsis E, et al. Immunotherapy for pancreatic cancer: A 2020 update. Cancer Treat Rev. 2020 Jun;86:102016.

65. Keilson JM, Knochelmann HM, Paulos CM, Kudchadkar RR, Lowe MC. The evolving landscape of immunotherapy in solid tumors. J Surg Oncol. 2021 Mar;123(3):798-806.

66. Liu Z, Liu X, Liang J, Liu Y, Hou X, Zhang M, et al. Immunotherapy for Hepatocellular Carcinoma: Current Status and Future Prospects. Front Immunol. 2021;12:765101.

67. He X, Xu C. Immune checkpoint signaling and cancer immunotherapy. Cell Res. 2020 Aug;30(8):660-69.

68. Sangro B, Gomez-Martin C, de la Mata M, Inarrairaegui M, Garralda E, Barrera P, et al. A clinical trial of CTLA-4 blockade with tremelimumab in patients with hepatocellular carcinoma and chronic hepatitis C. J Hepatol. 2013 Jul;59(1):81-8.

69. Singal AG, El-Serag HB. Hepatocellular Carcinoma From Epidemiology to Prevention: Translating Knowledge into Practice. Clin Gastroenterol Hepatol. 2015 Nov;13(12):2140-51.

70. Papatheodoridis GV, Lampertico P, Manolakopoulos S, Lok A. Incidence of hepatocellular carcinoma in chronic hepatitis B patients receiving nucleos(t)ide therapy: a systematic review. J Hepatol. 2010 Aug;53(2):348-56.

71. Hosaka T, Suzuki F, Kobayashi M, Seko Y, Kawamura Y, Sezaki H, et al. Long-term entecavir treatment reduces hepatocellular carcinoma incidence in patients with hepatitis B virus infection. Hepatology. 2013 Jul;58(1):98-107.

72. Sawada Y, Yoshikawa T, Ofuji K, Yoshimura M, Tsuchiya N, Takahashi M, et al. Phase II study of the GPC3-derived peptide vaccine as an adjuvant therapy for hepatocellular carcinoma patients. Oncoimmunology. 2016 May;5(5):e1129483.

73. Toyoda H, Kumada T, Tada T, Kaneoka Y, Maeda A, Kanke F, et al.

Clinical utility of highly sensitive Lens culinaris agglutinin-reactive alpha-fetoprotein in hepatocellular carcinoma patients with alpha-fetoprotein <20 ng/mL. Cancer Sci. 2011 May;102(5):1025-31.

74. Zhang Z, Zhang Y, Wang Y, Xu L, Xu W. Alpha-fetoprotein-L3 and Golgi protein 73 may serve as candidate biomarkers for diagnosing alpha-fetoprotein-negative hepatocellular carcinoma. Onco Targets Ther. 2016;9:123-9.

75. Wan HG, Xu H, Gu YM, Wang H, Xu W, Zu MH. Comparison osteopontin vs AFP for the diagnosis of HCC: a meta-analysis. Clin Res Hepatol Gastroenterol. 2014 Dec;38(6):706-14.

76. Ji J, Wang H, Li Y, Zheng L, Yin Y, Zou Z, et al. Diagnostic Evaluation of Des-Gamma-Carboxy Prothrombin versus alpha-Fetoprotein for Hepatitis B Virus-Related Hepatocellular Carcinoma in China: A Large-Scale, Multicentre Study. PLoS One. 2016;11(4):e0153227.

77. Feng H, Li B, Li Z, Wei Q, Ren L. PIVKA-II serves as a potential biomarker that complements AFP for the diagnosis of hepatocellular carcinoma. BMC Cancer. 2021 Apr 13;21(1):401.

78. Dai M, Chen X, Liu X, Peng Z, Meng J, Dai S. Diagnostic Value of the Combination of Golgi Protein 73 and Alpha-Fetoprotein in Hepatocellular Carcinoma: A Meta-Analysis. PLoS One. 2015;10(10):e0140067.

79. Dong M, Chen ZH, Li X, Li XY, Wen JY, Lin Q, et al. Serum Golgi protein 73 is a prognostic rather than diagnostic marker in hepatocellular carcinoma. Oncol Lett. 2017 Nov;14(5):6277-84.

80. Fu Y, Xu X, Huang D, Cui D, Liu L, Liu J, et al. Plasma Heat Shock Protein 90alpha as a Biomarker for the Diagnosis of Liver Cancer: An Official, Large-scale, and Multicenter Clinical Trial. EBioMedicine. 2017 Oct;24:56-63.

81. Wu XM, Xi ZF, Liao P, Huang HD, Huang XY, Wang C, et al. Diagnostic and prognostic potential of serum microRNA-4651 for patients with hepatocellular carcinoma related to aflatoxin B1. Oncotarget. 2017 Oct 6;8(46):81235-49.

82. Lin XJ, Chong Y, Guo ZW, Xie C, Yang XJ, Zhang Q, et al. A serum microRNA classifier for early detection of hepatocellular carcinoma: a multicentre, retrospective, longitudinal biomarker identification study with a nested case-control study. Lancet Oncol. 2015 Jul;16(7):804-15.

83. Luo P, Yin P, Hua R, Tan Y, Li Z, Qiu G, et al. A Large-scale, multicenter serum metabolite biomarker identification study for the early detection of hepatocellular carcinoma. Hepatology. 2018 Feb;67(2):662-75.

84. Qiu F, Qiu F, Liu L, Liu J, Xu J, Huang X. The Role of Dermcidin in the Diagnosis and Staging of Hepatocellular Carcinoma. Genet Test Mol Biomarkers. 2018 Apr;22(4):218-23.

85. Shu QH, Ge YS, Ma HX, Gao XQ, Pan JJ, Liu D, et al. Prognostic value of polarized macrophages in patients with hepatocellular carcinoma after curative resection. J Cell Mol Med. 2016 Jun;20(6):1024-35.

86. Yao W, He JC, Yang Y, Wang JM, Qian YW, Yang T, et al. The Prognostic Value of Tumor-infiltrating Lymphocytes in Hepatocellular Carcinoma: a Systematic Review and Meta-analysis. Sci Rep. 2017 Aug 8;7(1):7525.

87. Mao CS, Yin H, Ning HB, Peng Z, Li K, Ding GQ. Levels of HBx, VEGF, and CEACAM1 in HBV-related hepatocellular carcinoma and their correlation with cancer prognosis. Eur Rev Med Pharmacol Sci. 2017 Oct;21(17):3827-33.

88. Dong ZZ, Yao DF, Yao M, Qiu LW, Zong L, Wu W, et al. Clinical impact of plasma TGF-beta1 and circulating TGF-beta1 mRNA in diagnosis of hepatocellular carcinoma. Hepatobiliary Pancreat Dis Int. 2008 Jun;7(3):288-95.

89. Yamashita T, Honda M, Nakamoto Y, Baba M, Nio K, Hara Y, et al. Discrete nature of EpCAM+ and CD90+ cancer stem cells in human hepatocellular carcinoma. Hepatology. 2013 Apr;57(4):1484-97.

90. Bruix J, Cheng AL, Meinhardt G, Nakajima K, De Sanctis Y, Llovet J. Prognostic factors and predictors of sorafenib benefit in patients with hepatocellular carcinoma: Analysis of two phase III studies. J Hepatol. 2017 Nov;67(5):999-1008.

91. Briggs A, Daniele B, Dick K, Evans TRJ, Galle PR, Hubner RA, et al. Covariate-adjusted analysis of the Phase 3 REFLECT study of lenvatinib versus sorafenib in the treatment of unresectable hepatocellular carcinoma. Br J Cancer. 2020 Jun;122(12):1754-59.

92. Vogel A, Qin S, Kudo M, Su Y, Hudgens S, Yamashita T, et al. Lenvatinib versus sorafenib for first-line treatment of unresectable hepatocellular carcinoma: patient-reported outcomes from a randomised, open-label, non-inferiority, phase 3 trial. Lancet Gastroenterol Hepatol. 2021 Aug;6(8):649-58.

93. Turpin A, de Baere T, Heurgue A, Le Malicot K, Ollivier-Hourmand I, Lecomte T, et al. Liver transarterial chemoembolization and sunitinib for unresectable hepatocellular carcinoma: Results of the PRODIGE 16 study. Clin Res Hepatol Gastroenterol. 2021 Mar;45(2):101464.

94. Faivre S, Raymond E, Boucher E, Douillard J, Lim HY, Kim JS, et al. Safety and efficacy of sunitinib in patients with advanced hepatocellular carcinoma: an open-label, multicentre, phase II study. Lancet Oncol. 2009 Aug;10(8):794-800.

95. Govindarajan R, Siegel E, Makhoul I, Williamson S. Bevacizumab and erlotinib in previously untreated inoperable and metastatic hepatocellular carcinoma. Am J Clin Oncol. 2013 Jun;36(3):254-7.

96. Abou-Alfa GK, Borgman-Hagey AE, Kelley RK. Cabozantinib in Hepatocellular Carcinoma. N Engl J Med. 2018 Oct 4;379(14):1384-85.

97. Freemantle N, Mollon P, Meyer T, Cheng AL, El-Khoueiry AB, Kelley RK, et al. Quality of life assessment of cabozantinib in patients with advanced hepatocellular carcinoma in the CELESTIAL trial. Eur J Cancer. 2022 Jun;168:91-98.

98. Zhu AX, Baron AD, Malfertheiner P, Kudo M, Kawazoe S, Pezet D, et al. Ramucirumab as Second-Line Treatment in Patients With Advanced Hepatocellular Carcinoma: Analysis of REACH Trial Results by Child-Pugh Score. JAMA Oncol. 2017 Feb 1;3(2):235-43.

99. Llovet JM, Singal AG, Villanueva A, Finn RS, Kudo M, Galle PR, et al. Prognostic and Predictive Factors in Patients with Advanced HCC and Elevated Alpha-Fetoprotein Treated with Ramucirumab in Two Randomized Phase III Trials. Clin Cancer Res. 2022 Jun 1;28(11):2297-305.

100. Zhu AX, Finn RS, Kang YK, Yen CJ, Galle PR, Llovet JM, et al. Serum alpha-fetoprotein and clinical outcomes in patients with advanced hepatocellular carcinoma treated with ramucirumab. Br J Cancer. 2021 Apr;124(8):1388-97.

101. Finn RS, Merle P, Granito A, Huang YH, Bodoky G, Pracht M, et al. Outcomes of sequential treatment with sorafenib followed by regorafenib for HCC: Additional analyses from the phase III RESORCE trial. J Hepatol. 2018 Aug;69(2):353-58.

102. Teufel M, Seidel H, Kochert K, Meinhardt G, Finn RS, Llovet JM, et al. Biomarkers Associated With Response to Regorafenib in Patients With Hepatocellular Carcinoma. Gastroenterology. 2019 May;156(6):1731-41.

103. Zhu AX, Kudo M, Assenat E, Cattan S, Kang YK, Lim HY, et al. Effect of everolimus on survival in advanced hepatocellular carcinoma after failure of sorafenib: the EVOLVE-1 randomized clinical trial. JAMA. 2014 Jul 2;312(1):57-67.