REVIEW

Early diagnosis and therapeutic strategies for hepatocellular carcinoma: From bench to bedside

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Abstract

Hepatocellular carcinoma (HCC) is the fourth leading cause of cancer-related deaths worldwide. The prognosis of patients with HCC remains poor largely due to the late diagnosis and lack of effective treatments. Despite being widely used, alpha-fetoprotein serology and ultrasonography have limited diagnostic performance for early-stage HCC. The emergence of omics strategies has contributed to significant advances in the development of non-invasive biomarkers for the early diagnosis of HCC including proteins, metabolites, circulating tumor deoxyribonucleic acid, and circulating non-coding ribonucleic acid. Early diagnosis is beneficial to patients as it increases the proportion who can be treated with curative treatment, thus prolonging survival outcomes. Currently, multiple clinical trials involving locoregional, systemic therapies, and combinations of these modalities are changing therapeutic strategies for different

stage HCC. Success in several preclinical trials that involve immunotherapeutic innovations has created the potential to complement and enforce other treatment strategies in the future. This review summarizes the most recent advances in non-invasive early molecular detection, current therapy strategies, and potential immunotherapeutic innovations of HCC.

Key Words: Hepatocellular carcinoma; Early detection; Biomarker; Therapeutic strategies; Immunotherapeutic innovations

Core Tip: Long-term survival relies upon early diagnosis and timely treatment for patients with hepatocellular carcinoma (HCC). In this review, an update on the non-invasive early molecular detection and therapeutic strategies associated with HCC is discussed, focusing on omics-related biomarkers (*e.g.*, proteins, metabolites, circulating tumor deoxyribonucleic acid, circulating non-coding ribonucleic acid), locoregional and systemic therapies for treating different stages of HCC, as well as potential immunotherapeutic innovations (*e.g.*, adoptive cell transfer therapy).

INTRODUCTION

Hepatocellular carcinoma (HCC) accounts for the predominant type of primary liver cancer, ranking as the sixth most common malignancy and representing the fourth leading cause of cancer-related deaths worldwide[1]. According to annual estimates, liver cancer will cause more than 1 million deaths in 2030, with a 5-year survival of 18%[2]. Although early diagnosis and prompt treatment may improve long-term outcomes, most HCC patients are diagnosed with advanced stages of disease and face a generally poor prognosis with limited treatment options.

Therefore, the precise and early diagnosis are crucial for the management of HCC to facilitate the best chance of long-term patient survival. Despite being commonly used to screen high-risk populations, ultrasonography (US) remains a suboptimal method to detect HCC at an early stage, with a relatively low sensitivity of only 40%-50%[3]. Moreover, the accuracy of US is often influenced by the operator's clinical experience and various patient-related factors such as obesity[1]. In turn, much attention has been focused on the development of non-invasive, reproducible, and quantifiable biomarkers that may allow for earlier diagnosis and better therapeutic interventions. Over the past decade, multiple studies have noted the potential and clinical value of omics-related biomarkers as a possible alternative option to facilitate the non-invasive diagnosis of tumors (Figure 1).

Therapeutic options to treat HCC have substantially improved over the last several decades, especially with the advancements made in molecular targeted drugs and immunotherapies. Clinical trials regarding locoregional and systemic therapies have paved the way for further improvement in patient outcomes. Nevertheless, identifying the best therapeutic strategies for different stage HCC remains a challenge due to the high tumor heterogeneity.

In this review, we summarize the most recent advances in non-invasive early detection and treatment options for HCC.

NON-INVASIVE BIOMARKERS FOR EARLY DIAGNOSIS OF HCC

The emergence of omics strategies has recently led to significant progress in the non-
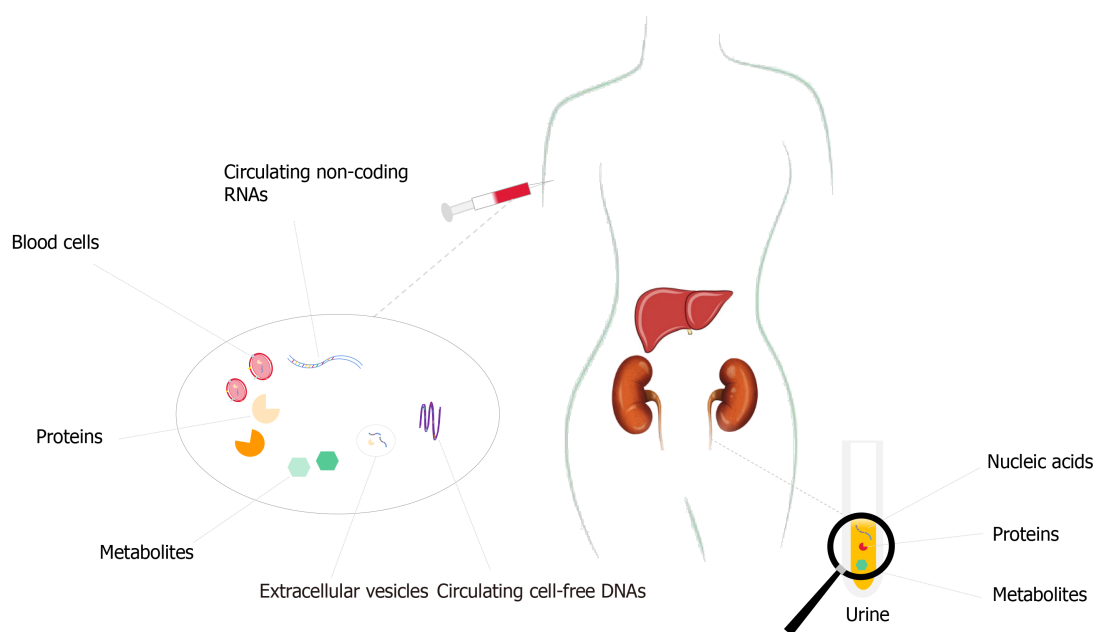
Figure 1 Non-invasive biomarkers for early diagnosis of hepatocellular carcinoma. For early diagnosis, prediction models combining protein biomarkers (*e.g.*, alpha-fetoprotein and Des-gamma carboxyprothrombin) with other parameters may soon be applied in clinical practice. These models hold the promise of high accuracy to identify early-stage hepatocellular carcinoma within high-risk populations such as individuals with cirrhosis or nonalcoholic steatohepatitis.

invasive early detection of HCC. These omics-related biomarkers are mainly derived from body fluid (*e.g.*, blood and urine) and include proteins, metabolites, circulating tumor DNA, and circulating non-coding RNA (Table 1).

Proteomics
Alpha-fetoprotein (AFP) and des-gamma-carboxy prothrombin (DCP) are the most widely accepted serological diagnostic biomarkers for HCC in clinical practice. The sensitivity and specificity of a single biomarker for the early diagnosis of HCC vary considerably, suggesting that a single biomarker to detect early-stage HCC is insufficient and has unsatisfactory diagnostic performance. In contrast, the combination of several biomarkers increases early diagnostic rates. The combination of biomarkers with other parameters (*e.g.*, sex and age) into prediction models to generate an aggregate score to help detect early HCC has been more promising, as demonstrated by such tools as the GALAD model, the ASAP model, and HES algorithm[4-6] (Table 2).

The GALAD model, which combines age and gender into an algorithm based on serum AFP, lens culinaris agglutinin-reactive fraction of AFP (AFP-L3), and DCP levels, was developed to determine the risk of HCC in high-risk individuals. Of note, the GALAD model has been validated in different cohorts from Germany, Japan, Hong Kong, and the United Kingdom[4]. Given its proper diagnostic value, Yang *et al*[7] compared the performance of the GALAD model *vs* the use of abdominal US to detect HCC. Interestingly, the GALAD score surpassed the ability of US to predict HCC with a sensitivity of 92%, specificity of 79%, and an area under curve (AUC) of 0.92 at a cut-off of-1.18, especially among HCC patients with Barcelona Clinic Liver Cancer (BCLC) stage 0-A. Given the increasing prevalence of nonalcoholic steatohepatitis (NASH)-based HCC and the lack of effective screening strategies for early-stage NASH-HCC, Best *et al*[8] estimated the performance of GALAD model among patients with early NASH-HCC without cirrhosis. To detect BCLC stage A NASH-HCC, the model had an AUC of 0.92 with 86.2% sensitivity and 90.9% specificity at a cut-off of-1.334. To identify stage A NASH-HCC without cirrhosis, the AUC value was 0.94 with 85.7% sensitivity and 96.2% specificity at a-0.63 cut-off. As such, the GALAD score may facilitate the surveillance of individuals with NASH, thereby helping to offset the limitation of US to detect HCC in this high-risk population.

The HES algorithm, which includes current AFP level, rate of AFP change, alanine aminotransferase level, platelet count, and age, was initially constructed to predict the development of HCC among patients with hepatitis C and cirrhosis[5]. The score was further validated in subsequent studies that evaluated the identification of HCC among patients with different etiologies of cirrhosis[9]. With a specificity of 90%, the

Table 1 Non-invasive biomarkers for early diagnosis of hepatocellular carcinoma

Type of biomarker	Examples
Proteins	AFP; DCP; GALAD score; HES algorithm; ASAP model
Metabolites	A panel based on phenylalanyl-tryptophan and glycocholate
Circulating cell-free DNAs	Somatic mutations; DNA methylation; 5-hydroxymethylcytosine
Circulating non-coding RNAs	Micro-RNAs (mirR-125b, miR-122, and miR-21); Circular RNAs; Long non-coding RNAs; Exosomal non-coding RNAs

AFP: Alpha-fetoprotein; DCP: Des-gamma-carboxy prothrombin; HCC: Hepatocellular carcinoma; RNA: Ribonucleic acid.

Table 2 Introduction of alpha-fetoprotein-based diagnostic models for hepatocellular carcinoma

Model	Parameters	Ref.	Notes
GALAD score	Gender, Age, AFP-L3, AFP, and DCP	[4,7,8]	Validated in different cohorts from Germany, Japan, Hong Kong, the United Kingdom; Surpassing the ability of ultrasound to predict HCC; Excelling in diagnosing early-stage HCC in the setting of cirrhosis or CHB or NASH
HES algorithm	Current level of AFP, rate of AFP change, level of alanine aminotransferase, platelet count, and age	[5,9,10]	Validated in the detection of HCC in patients with cirrhosis of any etiology; Superior to the AFP measuring alone in detecting early-stage HCC
ASAP model	Age, gender, AFP, and DCP	[6]	Validated in the presence of HCC in patients with CHB; Exhibiting 73.8% sensitivity and 90.0% specificity for detecting BCLC stage 0-A HCC

AFP: Alpha-fetoprotein; AFP-L3: Lens culinaris agglutinin-reactive fraction of AFP; BCLC: Barcelona clinic liver cancer; CHB: Chronic hepatitis B; DCP: Des-gamma-carboxy prothrombin; HCC: Hepatocellular carcinoma; NASH: Nonalcoholic steatohepatitis.

HES algorithm was able to identify HCC with 52.56% sensitivity *vs* 48.13% for AFP alone ($P < 0.0005$) 6 mo prior to ultimate diagnosis. After incorporating the etiologic cause of cirrhosis, the updated HES remained superior to AFP alone to detect early-stage HCC with nearly no additional cost and the sensitivity improved by 5.18% ($P = 0.0015$)[10]. The ASAP, which incorporates age, gender, serum AFP and DCP levels, was another model developed to predict the occurrence of HCC in patients with chronic hepatitis B[6]. To detect BCLC stage 0-A HCC, the ASAP reportedly has 73.8% sensitivity and 90.0% specificity. Despite this, the best model with highest diagnostic accuracy remains controversial, due to the complexity and heterogeneity of HCC related to different etiologies, environmental, and genetic factors.

To improve the early detection rate of HCC, many studies have recently evaluated the diagnostic performance of other protein biomarkers such as plasma heat shock protein 90alpha[11], serum aldo-keto reductase family 1 member B10[12], and site-specific N-glycopeptides in serum haptoglobin[13]. In addition, urinary proteomic analysis has also gradually been increasingly investigated as a possible means to obtain biomarkers for HCC screening, diagnosis, and surveillance[14,15]. Unfortunately, to date, none of these protein biomarkers have been utilized in clinical practice or recommended by professional associations, highlighting the challenges and present status of biomarker development.

Metabolomics
Metabolomics plays a vital role in providing insights into the etiology and mechanisms of HCC. In turn, metabolites in body fluids, which could directly and uniquely reflect the metabolic changes in the liver, may have the potential to become novel candidate markers for early diagnosis of HCC. To this point, analyzing plasma components, Di Poto *et al*[16] identified 11 metabolites and combined these metabolites with three clinical variables to propose a diagnostic strategy to detect early-stage HCC among patients with liver cirrhosis. Luo *et al*[17] discovered and validated another serum metabolite biomarker panel, which consisted of phenylalanyl-tryptophan and glycocholate; the panel demonstrated a good diagnostic performance for early detection of HCC among high-risk populations. In addition to identifying small or AFP false-negative HCCs, the panel had high sensitivity ranging from 71.4% to 80.0% to detect "preclinical" HCC from 1 to 3 years before a confirmed HCC diagnosis. Kim *et al*[18] constructed another prediction model, which included methionine, proline, ornithine, pimelylcarnitine, and octanoylcarnitine, that demonstrated excellent ability

to detect early HCC. While several studies have investigated plasma metabolites, high-quality studies are lacking on the use of urinary metabolites. Future large prospective studies are needed to validate whether metabolite-based panels can improve current HCC surveillance and management strategies.

Circulating cell-free DNA

Circulating cell-free DNAs (cfDNAs) are composed of extracellular DNA fragments shed into the blood *via* any moribund normal or malignant cells[19]. Circulating tumor DNA (ctDNA), which accounts for a fraction of cfDNA, carries tumor-specific genetic or epigenetic variation, such as genetic mutations and DNA methylation. Several studies have suggested that ctDNA can assist in cancer screening and diagnostic strategies through the use of non-invasive liquid biopsies (*i.e.* molecular testing of solid malignancies *via* blood analysis). To date, a variety of tumor-specific genetic and/or epigenetic changes have been detected that may be promising ctDNA-based biomarkers for the diagnosis of early HCC. In particular, the combined detection of cfDNA somatic mutations and protein markers has the potential to identify early-stage HCC from asymptomatic HBsAg-seropositive individuals[20]. A recent systematic review, which included 10 studies reporting on different biomarker combinations, demonstrated that the combined analysis of cfDNA and serum AFP level yielded better detection rates of HCC than AFP alone[21].

To date, DNA methylation profiling of ctDNA has been the most promising approach for early identification of HCC among high-risk patients[22]. Given that the septin 9 (SEPT9) promoter in cfDNA is hypermethylated in HCC, methylated SEPT9 testing has been proposed as a useful blood epigenetic biomarker for HCC diagnosis among cirrhotic patients[23]. Xu *et al*[24] developed a diagnostic prediction model based on ten methylation markers in ctDNA to differentiate between HCC and benign liver diseases; this model had high diagnostic specificity and sensitivity (both *P* < 0.001). Kisiel *et al*[25] constructed a novel panel based on six plasma methylated DNA markers for HCC detection, which was significantly superior to AFP alone. In the phase II clinical validation trial that included 95 HCC patients, 51 cirrhotic controls, and 98 healthy volunteers, the panel had an AUC of 0.96 with a sensitivity of 75% for BCLC stage 0 HCC and a sensitivity of 93% for stage A HCC. A separate study demonstrated that combined assays of blood methylated DNA markers and serum AFP values had a superior capacity to distinguish HCC individuals from healthy subjects *vs* AFP alone[26]. Specifically, this panel, which included three methylated DNA markers (homeobox A1, empty spiracles homeobox 1, and testis-specific Y-encoded-like protein 5), beta-1,3-galactosyltransferase 6, and two protein markers (AFP and AFP-L3), had a sensitivity of 71% and a 90% specificity to diagnose early-stage HCC, which was superior to the GALAD score or AFP alone. Thus, the analysis of ctDNA can serve as a complementary strategy coupled with AFP testing for early HCC detection.

Besides alterations in methylation patterns, another epigenetic change in cfDNA, 5-hydroxymethylcytosine (5hmC), may facilitate early diagnosis of cancer[27,28]. 5hmC is not only associated with active DNA demethylation, but also has a relatively stable DNA epigenetic signature[28]. Cai *et al*[29] constructed a diagnostic model based on 5hmC markers identified in cfDNA and exhibited high capability for differentiating patients with early HCC from individuals with chronic hepatitis B virus (HBV) infection or cirrhosis, as well as from those with benign liver diseases and healthy controls. Taken together, the assays of cfDNAs, including ctDNA in body fluids, may provide a solid foundation for early detection and precise cancer diagnosis. More large-scale studies are required for further validation of these conclusions.

Circulating non-coding RNA

Data have suggested a regulatory role of non-coding RNAs (ncRNAs) in the development and progression of HCC[30]. A rapidly growing area of basic research given the advancements in sequencing techniques in recent years, circulating ncRNAs research has identified this area as ncRNAs as potential molecular biomarkers to diagnose cancer. In particular, microRNAs (miRNAs), circular RNAs (circRNAs), and long non-coding RNAs (lncRNAs) have increasingly attracted considerable attention.

miRNAs, a class of single-stranded ncRNAs with about 20 nucleotides length, mainly function as regulatory RNAs that control gene expression at the post-transcriptional levels. Several circulating miRNAs have already been identified to be relative to HCC, with different diagnostic values. One study demonstrated that miR-3197 had the potential to identify individuals at risk of HCC during the period of anti-viral treatment, since its expression levels in serum varied significantly with disease progression[31]. Besides, mirR-125b[32], miR-122[33,34], and miR-21[35] may serve as promising biomarkers for the early diagnosis of HCC. Subgroup analysis in a recent

meta-analysis noted that the pooled sensitivity, specificity, and AUC of miR-125b for detecting HBV-related HCC patients were 0.95, 0.79, and 0.95, respectively[32]. Another meta-analysis of 13 studies including 920 HCC patients and 1217 controls revealed that the pooled sensitivities, specificities, and AUCs of serum miR-122 were 0.76, 0.75, and 0.82 for distinguishing HCC patients from overall controls; and 0.79, 0.82, and 0.87 for differentiating HCC from HBV or hepatitis C virus infection; respectively[33]. MiR-21 might be another useful biomarker for early detection of HCC, with the pooled sensitivity, specificity, and AUC of 85.2%, 79.2%, and 89%, respectively[35]. However, prospective population-based studies with larger sample sizes and different ethnic groups are needed to further validate these findings.

The diagnostic performance of a single miRNA biomarker may vary considerably, however, among different studies. Like cfDNAs, miR-panels are likely to improve the specificity and sensitivity of HCC diagnosis by integrating multiple miRNAs and some clinical parameters. For example, combining miR-27b-3p and miR-192-5p into a panel yielded considerable higher diagnostic value to detect HCC, especially among patients with AFP-negative tumors[36]. Of note, another miRNA panel that consisted of seven serum miRNAs (miR-29a, miR-29c, miR-133a, miR-143, miR-145, miR-192, and miR-505) was constructed by Lin *et al*[37] using multicenter data. This miRNA panel performed well to detect small HCC, early-stage HCC, and AFP-negative HCC among high-risk individuals, thus contributing to HCC screening and surveillance[37]. In a recent meta-analysis that compared the diagnostic effectiveness of single miRNAs and miRNA panels, the authors noted that combining circulating miRNAs and AFP performed better to diagnosis HCC; miRNAs did, however, have higher accuracy than AFP alone for early detection of HCC[38].

Extracellular vesicles (EVs), including exosomes, microvesicles, and apoptotic bodies, are small vesicular bodies with phospholipid bilayer membrane structure that are released into body fluid by various cells[39]. EVs usually capsulize miRNAs, lncRNAs, and other small molecules, to protect them from degradation. EVs and these molecules are involved in facilitating tumor development and progression. In turn, exosomal miRNAs have been investigated as potential diagnostic biomarkers and therapeutic targets for HCC. For the early detection of HCC, serum exo-miR-10b-5p has been reported to be a promising biomarker, with 90.7% sensitivity and 75.0% specificity at the cut-off value of 1.8-fold[40]. Notably, a novel panel comprising exo-miR-4661-5p and exomiR-4746-5p yielded a best fit AUC of 0.947 with a sensitivity of 75% and a specificity of 91.7% for early-stage HCC[41]. A separate study reported that the combination of exo-miR-122, exo-miR-148a, and AFP was able to distinguish early HCC from cirrhosis with an AUC of 0.931[42]. In addition, mounting evidence also has indicated that lncRNAs with a length of > 200 nucleotides and circRNAs with a covalently closed loop structure could serve as novel diagnostic biomarkers for early-stage HCC such as serum EV-derived lncRNA LINC00853[43] and LINC00853[43], and plasma circRNA panels[44].

ADVANCES IN CURRENT THERAPEUTIC STRATEGIES

Liver resection

Liver resection (LR) is a commonly used curative approach for eligible early-stage HCC individuals. Compared with other solid organs, the liver is characterized by an extremely strong regenerating capacity that can facilitate a return to a fully functional mass in a short time after losing major tissue[45]. The applied definitions of tumor resectability have varied among different institutions and are debated. Generally, LR is applicable to patients with no more than three lesions, and without vascular invasion or extrahepatic metastases[46]. Moreover, compared with other alternative treatments, LR can provide a survival benefit for patients with BCLC stage B/C HCCs[47], as well as individuals with portal vein tumor thrombus[48]. Although LR can achieve a 5-year overall survival (OS) of 60%-70%[49], the high incidence of HCC recurrence following LR remains a major challenge. Among patients with intrahepatic recurrence or extrahepatic recurrence after resection, re-LR may improve long-term survival[50].

More recently, minimally invasive approaches to LR have been increasingly adopted due to proven safety and effectiveness reported in many studies. Specifically, in two recent meta-analyses, laparoscopic LR achieved equivalent long-term oncological outcomes and exhibited excellent short-term clinical advantages compared with open LR, with less intraoperative blood loss and blood transfusions, fewer complications, and a shorter hospital stay[51,52]. Even among patients with post-hepatectomy recurrent HCC, laparoscopic LR may be a safe and effective treatment

option[53]. Nevertheless, there is still a lack of large-scale randomized controlled trials to evaluate the superiority of either the open *vs* minimally invasive approach.

Liver transplantation

Liver transplantation (LT) represents the best therapeutic option for early-stage unresectable HCC within the Milan criteria (MC) for many patients. In particular, LT can provide a 10-year recurrence-free survival (RFS) of 50%-70%[54]. MC (single lesion < 5 cm, or multiple lesions with less than 3 nodules and a maximum diameter of less than 3 cm) has long been established as the standard criteria for LT patient selection. Over the last decade, there has been controversy regarding the expansion of current MC for liver transplantation. Some data have noted that patients meeting expanded criteria had equivalent post-transplant survival outcomes compared with individuals within MC[55]. Given that locoregional treatment has been shown to effectively downstage HCCs from beyond to within MC, Mazzaferro *et al*[56] conducted a randomized controlled phase IIb/III trial to determine the efficacy of LT among patients who achieved clinical downstaging of HCC beyond MC. As expected, LT improved tumor event-free survival and OS compared with non-transplantation therapies. Another multicenter study also demonstrated that effective clinical downstaging resulted in favorable post-LT outcomes[57]. Thus, the practice of downstag-ing therapies before LT may be beneficial for the expansion of patient selection. Early recurrence after LT (< 12 mo) is usually associated with a poor prognosis, but systemic or locoregional treatments may provide treatment options for these patients[58].

Ablation therapy

Ablation therapy is another commonly used treatment for patients with HCC ≤ 3 cm in size. Radiofrequency ablation (RFA) and microwave ablation (MWA), the two most common types of ablation therapy, are mainly performed using radiofrequency or microwave-generated thermal effects to cause local tumor necrosis[59]. A Japanese study that reported on actual 10-year survival after MWA for HCC noted that median OS, recurrence-free survival, and 10-year OS was 5.5 years, 2.4 years, and 23.8%, respectively[60]. Of note, among patients with lesions less than or equal to 4 cm in diameter, both MWA and RFA achieved similar therapeutic effects[61].

Given that the therapeutic efficacy of RFA and LR varied according to tumor size, Zheng *et al*[62] compared clinical outcomes after RFA and LR for HCC less than 5 cm. Interestingly, both RFA and LR achieved comparable OS and cancer-specific survival for HCC patients with lesions less than 3 cm, but LR was associated with increased long-term survival among patients with HCC ranging from 3.1 to 5 cm in diameter. A recent meta-analysis demonstrated, however, that LR provided more survival benefits, including better RFS and lower local recurrence compared with RFA[63]. Generally, ablation alone is not recommended for lesions > 5 cm, but MWA can achieve a desirable radical curative efficacy after downstaging by other therapies[64].

The introduction of advanced stereotactic navigation technology into ablation approaches has further expanded its application and scope. Stereotactic radiofrequency ablation has been reported to be an effective therapeutic option for HCC, even if the lesion size is > 3 cm[65]. Of note, stereotactic microwave ablation was safe and feasible among HCC patients, with acceptable short- and long-term clinical outcomes, particularly for tumors with complex anatomic locations[66,67].

Radiation therapy

Traditionally, radiation therapy (RT) had a limited role in HCC treatment. According to present guidelines, RFA, and not RT, is recommended as first-line therapy for unresectable early-stage HCC. Of note, stereotactic body radiation therapy (SBRT) is a highly conformal technique that enables delivering high doses of radiation to targeted lesions under high-precision image guidance. More studies have suggested that SBRT may be well-tolerated and effective treatment alternative for patients with unresectable HCC[68,69], especially for subphrenic tumors more than 3 cm in diameter, or lesions with progression after transarterial chemoembolization (TACE)[70]. For patients with intrahepatic recurrent HCC, repeat SBRT has yielded promising oncologic effects with a median OS of 71 mo for the 1st SBRT *vs* 44 mo for the 2nd SBRT[71]. A recent meta-analysis also confirmed that SBRT could achieve better local control for HCC *vs* RFA, but OS with SBRT was significantly shorter compared with RFA, warranting further investigation[72].

Transcatheter arterial chemoembolization

Conventional TACE is recommended as standard therapy for intermediate-stage HCC. TACE generally involves the mixture of chemotherapeutic agents (*e.g.*, doxorubicin or cisplatin) and an embolic material (*e.g.*, lipiodol) injected into the target artery *via* a catheter[73]. The efficacy and safety of lipiodol-based TACE were the topic of a systematic review with a reported objective response rate (ORR), 1-year OS, 3-year OS, 5-year OS, median OS, and overall mortality of 52.5%, 70.3%, 40.4%, 32.4%, 19.4 mo, and 0.6%, respectively[74]. An elevation in post-treatment transaminase levels and postembolization syndrome are typically the most frequently observed complications[74]. Prophylactic use of dexamethasone can reduce the occurrence of TACE-related fever, anorexia, and nausea[75]. cTACE has also been examined as an adjuvant therapy among patients at high-risk of recurrence after resection of HBV-related HCC[76]. Additionally, TACE with drug eluting beads has been reported to achieve comparable results as cTACE, but with lower systemic toxicity[73]. A prospective single-arm phase II study reported the efficacy of TACE with drug eluting beads using idarubicin for unresectable HCC; this study noted a 6-mo ORR, median progression-free survival (PFS), and median OS of 52%, 6.6 mo, and 18.6 mo, respectively[77]. The presence of macroscopic vascular invasion has been associated with poor prognosis among patients with advanced HCCs. Several studies noted that combination therapy of TACE and RT was able to achieve better survival outcomes in these patients compared with TACE plus systemic therapy[78,79].

Systemic therapy

After a decade of negative clinical trials, systemic therapy with sorafenib, an oral small molecule multikinase inhibitor, was approved as a standard first-line treatment option for advanced HCC in 2007. Prospective data demonstrated that sorafenib delayed the progression of the disease and prolonged patient survival compared with placebo[80]. Multiple clinical trials investigating the efficacy of novel molecular targeted drugs have subsequently failed to exhibit superiority or non-inferiority to sorafenib until the recent emergence of another multikinase inhibitor, lenvanitib. In 2018, lenvatinib was demonstrated to yield a similar survival time as sorafenib in treatment of patients with advanced HCC (median OS, 13.6 mo *vs* 12.3 mo) in a randomized phase III trial[81]. Since that study, additional therapeutic strategies for advanced HCC have evolved. Specifically, lenvatinib is now recommended as the first-line treatment by several clinical guidelines, and another two multikinase inhibitors (*i.e.* regorafenib and cabozantinib) are recommended as second-line options. A meta-analysis demonstrated that regorafenib was associated with a median OS, median PFS, as well as pooled objective response rate of 11.08 mo, 3.24 mo, and 10.1%, respectively[82].

Given that angiogenesis can lead to tumor growth and metastasis and HCC is a typical blood-rich tumor, antiangiogenic inhibitors (*e.g.*, ramucirumab and bevacizumab) have been utilized in the clinic to treat advanced HCC. A systematic review and network meta-analysis of 14 trials revealed that regorafenib and cabozantinib were preferred treatment alternatives in refractory HCC patients, with ramucirumab as an additional option for individuals with high-AFP-producing HCCs (≥ 400 ng/mL)[83].

The clinical benefits of immunotherapeutic drugs for HCC have also been a topic of investigation. The implementation of a single programmed cell death protein 1/programmed death ligand 1 inhibitor (*e.g.*, nivolumab, pembrolizumab, camrelizumab, and atezolizumab) has been recommended as second-line therapy in advanced HCC. Three large-scale trials demonstrated that the median OS and ORR of nivolumab, pembrolizumab, or camrelizumab in patients with previously treated advanced HCC were 15.1, 13.9, and 13.8 mo, as well as 14.0%, 18.3%, and 14.7%, respectively[84-86]. Several investigations into the clinical efficacy of combining targeted drugs with immunotherapies have also been launched. In a phase Ib trial, lenvatinib combined with pembrolizumab had promising anti-tumor effects for unresectable HCC, with median OS of 22 mo and reasonable toxicity[87]. For patients with unresectable HCC who did not undergo systemic treatment previously, atezolizumab plus bevacizumab was associated with better OS and PFS than sorafenib[88]. Thus, this combination of therapy has recently become a promising first-line treatment option for patients with advanced HCC.

The combination of locoregional and systemic therapies has also gained attraction in the treatment of patients with intermediate- or advanced-stage HCC. To date, several studies have evaluated the efficacy of the combination of TACE and sorafenib compared with single therapy[89-91]. There is still no consensus, however, on whether this combination is superior for HCC patients. It is important to note that variations in treatment intervals between sorafenib and TACE may yield a different efficacy. Future

efforts should be directed toward defining the optimal combination of therapeutic approaches for unresectable HCC.

POTENTIAL IMMUNOTHERAPEUTIC INNOVATIONS

At present, the basic mechanisms of HCC-induced immunosuppression remain less investigated, but the immunotherapeutic strategies are mainly based upon two fundamental rules: the capability to release current immunologic responses; and the requirement to trigger other immunologic responses[92]. Accumulating evidence suggests that anti-tumor immunotherapy can be achieved by regulating the function or number of immune cells, immune receptors, or corresponding ligands, and so on.

Adoptive cell transfer therapy

Recent advancements in adoptive cell transfer therapy, an approach to deliver autologous engineered immunocytes, have brought new hope for patients with cancer. Generally, these modified cells include chimeric antigen receptor T cells (CAR-T), T cell receptor (TCR)-engineered T cells (TCR-T), and cytokine-induced killer cells (CIK). With the approval and clinical implementation of CD19-targeted CAR-T cells for acute lymphoblastic leukemia and lymphoma, this therapy also achieves excellent results in solid tumors, *e.g.*, HCC.

After modified by viral vectors, CAR-T cells are equipped with a positioning navigation device, *i.e.* an extracellular single-chain variable fragment of an antibody (scFv), specifically identifying tumor cells. Once CAR identifies tumor-associated antigens (TAA), T cell activation and proliferation is triggered by an intracellular signaling domain (CD3-ζ) and the additional costimulatory domains (*e.g.*, cluster of differentiation 28 [CD28], 4-1BB, and CD134). Then the activated T cells rapidly perform immune functions to kill targeted cells[93-95]. Unrestricted by major histocompatibility complex (MHC), CAR-T cells can directly recognize tumor cells and avoid the immune-evasion mechanism of tumor cells *via* down-regulation of MHC[96]. Given that glypican-3 (GPC3), a heparan sulfate proteoglycan located in the cell membrane, is rarely expressed in healthy liver tissue but overexpressed in HCC, it may serve as a promising tumor antigen targeted by CAR-T[97]. To this point, GPC3-CAR-T therapy has been the focus of several recent clinical trials. In a phase I trial of GPC3-specific CAR-T for 13 advanced-stage HCC patients with lymphodepletion induced by chemotherapy, Shi *et al*[98] demonstrated that it was a safe, well-tolerated, and practical approach, with 1- and 3-year OS of 42.0% and 10.5% with reasonable toxic side effects. In contrast, in mouse experiments, a soluble programmed cell death 1-chimeric 3 [PD1-CH3] fusion protein derived from PD1 and IgG4 was introduced into GPC3-CAR-T and exhibited enhanced anti-tumor activities[99]. Transgenic expression of interleukin 15 (IL-15) and IL-21 also elevated the anti-tumor capability of GPC3-CAR-T against HCC *in vivo*[100]. GPC3- or NKG2D-based CAR natural killer cells may also be a promising immunotherapeutic target for HCC[101,102].

Given that most tumor antigens are not expressed on the cell membrane but in the cytoplasm, TCR-T cells, genetically modified with TCRs specific for TAAs, are able to recognize specifically tumor antigen peptides presented by MHC, which overcomes the deficiency of tumor surface antigens[94]. Several HCC-related antigens targeted by TCR have been identified, *e.g.*, AFP-, GPC3-, virus-, NY-ESO-1-, and hTERT-specific TCRs[95]. Despite the success of NY-ESO-1-targeted TCR-T cells to treat multiple myeloma and synovial sarcoma, recent studies about TCR-T for HCC focus on targeting AFP[95]. The generation of affinity-optimized TCRs with specificity to AFP-positive tumors calls for the identification of novel AFP peptides; for example, the construction of HLA-A2/AFP$_{158}$-specific TCRs and HLA-A24/AFP$_{2-11}$-specific TCR forms the foundation to develop effective and safe TCR-T cells-based immunotherapies[103-105].

CIK cells are generated by culturing T lymphocytes with cytokines *in vitro*. The primary effector cells are CD3+/CD56+ T cells, which integrate the dual function of both T cells and natural killer cells. As adjuvant immunotherapy, CIK therapy has achieved remarkable results for HCC treatment in several recent clinical trials. In a phase III trial, Lee *et al*[106] demonstrated that adjuvant CIK therapy improved RFS and OS among patients after curative resection for HCC, with median RFS of 44.0 mo and OS not reached. After an extended 5-year follow-up, the superiority of this adjuvant immunotherapy for HCC was further estimated, with marked improvement in RFS and OS and lower risk of recurrence or death[107]. However, not all patients responded to this therapy. Yu *et al*[108] noted that targeting myeloid-derived suppressor cells might

be an effective therapeutic strategy to enhance the anti-tumor efficacy of CIKs for HCC treatment. With more in-depth research and more robust clinical trials ongoing, CIK therapy will be an appealing strategy to treat HCC.

Immune checkpoint inhibitors

Immune checkpoints are regulatory molecules involved with inhibitory receptors and signaling pathways, which can inhibit the function of T cells under normal physiological conditions, thereby allowing tumors to escape from host immune system and avoid being eliminated. In addition to cytotoxic T-lymphocyte-associated antigen 4 and PD-1 and its ligands, other inhibitory checkpoint molecules have also been identified (*e.g.*, T-cell immunoglobulin and mucin-domain containing-3, lymphocyte-activation gene 3, B and T lymphocyte attenuator, and T cell immunoreceptor with Ig and ITIM domains [TIGIT]). Recently, Chiu *et al*[109] reported that tumor resistance to PD-1 inhibitors in nude mouse xenograft model was mediated by inhibitory Poliomyelitis receptor associated protein 1 antibody/pulmonary vascular resistance/TIGIT axis, indicating that TIGIT was a promising target molecule to reverse the anti-PD1 resistance. In contrast, Ostroumov *et al*[110] demonstrated that overexpression of TIGIT was associated with the process of T cell exhaustion in liver cancer. The combination of its antibody with the PD1 inhibitor may effectively hinder the growth of liver cancer in immunocompetent mice. T-cell immunoglobulin and mucin-domain containing-3 was also involved in hepatocarcinogenesis, such as mediating effector T cell exhaustion[111].

Tumor vaccination

Vaccines derived from tumor antigen peptides or dendritic cells allow for activation of the immune system to eliminate tumors. GPC3-derived vaccine was previously studied and the results of a phase II study involving the vaccine as adjuvant therapy for HCC were published in 2016. The study demonstrated that GPC3-peptide vaccine reduced the risk of 1-year recurrence among patients with GPC3-positive HCC[112]. On long-term follow-up, this adjuvant therapy provided better survival outcomes by activating cytotoxic T lymphocyte[113]. More preclinical studies to optimize GPC3 vaccine are currently being conducted. Recently, XCL1-GPC3 fusion molecules were constructed as a novel HCC vaccine by integrating the XCL1 chemokine with GPC3, which can stimulate the activation of immune system in mice and have a synergistic anti-tumor effect with anti-PD-1[114]. Dendritic cells (DCs), as potent antigen-presenting cells, are a weapon for killing cancer cells; in turn, DC vaccines may be a favorable potential immunotherapy for HCC. A phase II study of adjuvant therapy with autologous dendritic cells for HCC has been completed by Lee *et al*[115]. This study noted reduction in the risk of tumor recurrence among HCC patients who received standard therapies with autologous DC[115]. Concerning combination therapy of DC vaccine and programmed death-ligand 1 inhibitor, Teng *et al*[116] also noted an excellent anti-tumor effect in mice, with longer OS and smaller tumor size.

Oncolytic virus

Oncolytic virus therapy is another emerging treatment approach, with viral vectors (*e.g.*, adenovirus, reovirus, M1 virus, and herpes virus) loading anti-cancer oncogenes. Owing to the risk of systemic adverse reactions, the delivery of these viruses to targeted lesions remains a major challenge. Yoon *et al*[117] constructed mesenchymal stem cells with the characteristic of tumor-homing as a promising carrier to deliver targeted modified oncolytic adenovirus safely into tumors to enhance anti-HCC efficacy. Lin *et al*[118] reported that oncolytic herpes simplex virus with a single-chain variable fragment against PD1 could change the immunosuppressive tumor microenvironment in HCC and elevate the anti-tumor effect.

CONCLUSION

In this review, we summarized the latest research progress in non-invasive biomarkers for early diagnosis of HCC (*e.g.*, proteins, metabolites, circulating cfDNAs, and ncRNAs), current therapeutic strategies, and potential immunotherapeutic innovations. For early diagnosis (Figure 1), prediction models combining protein biomarkers (*e.g.*, AFP and DCP) with other parameters may soon be applied in clinical practice. These models hold the promise of high accuracy to identify early-stage HCC within high-risk populations such as individuals with cirrhosis or NASH. The utilization of currently available data in the clinic not only enables reducing additional

healthcare costs, but also facilitates physicians making an objective and accurate diagnosis. In particular, cfDNA methylation scores to facilitate early detection of HCC in high-risk individuals seem to be the most promising strategy for early diagnostic workup rather than traditional HCC surveillance approaches (*i.e.* US with or without AFP). Meanwhile, miRNAs and metabolites have also exhibited great diagnostic potential to detect early-stage HCC. Candidate biomarkers or panels that enable identification of tumors within 6-12 mo before imaging examinations will provide more early curative treatment opportunities for patients. However, several limitations exist. Many studies to date have lacked appropriate study populations and enough sample size. Specifically, comparisons between early HCC patients and high-risk individuals with cirrhosis or with NAFLD, rather than any stage HCC and the healthy subjects, are needed. In addition, the establishment of standardization to collect, handle, storage, and analytic methods are required to ensure consistency in clinical application.

Regarding therapeutic strategies (Figure 2), patients with early-stage HCC should be elevated for curative treatments including hepatectomy, liver transplantation, or ablation depending on the patient performance status, underlying liver function, as well as disease-specific characteristics. Although there remains some controversy surrounding treatment options for intermediate-stage HCC, based on the latest evidence, TACE with or without sorafenib combination is likely the most appropriate strategy for unresectable HCCs. As for advanced-stage HCC, sorafenib, or a combination of atezolizumb and bevacizumab is the preferred treatment strategy. In addition, results from clinical trials involving systemic therapies or combinations of locoregional and systemic therapies have suggested these approaches may be effective for intermediate- and advanced-stage HCC. Also, adoptive cell transfer therapy, tumor vaccination, and oncolytic virus may complement or even change current treatment strategies for HCC in the future, especially CAR-T and CIK therapy. The success of CAR-T therapy for HCC is, however, hindered by the selection of TAA, an efficient migration and infiltration of CAR-T into the tumor lesions, as well as the survivability of CAR-T in the tumor microenvironment, which urgently needs to be solved[119].

In conclusion, the early diagnosis and multidisciplinary collaboration will promote the ongoing emergence of novel therapies for HCC. Future well-designed clinical trials are needed to improve early detection of HCC and thus contribute to a better prognosis.
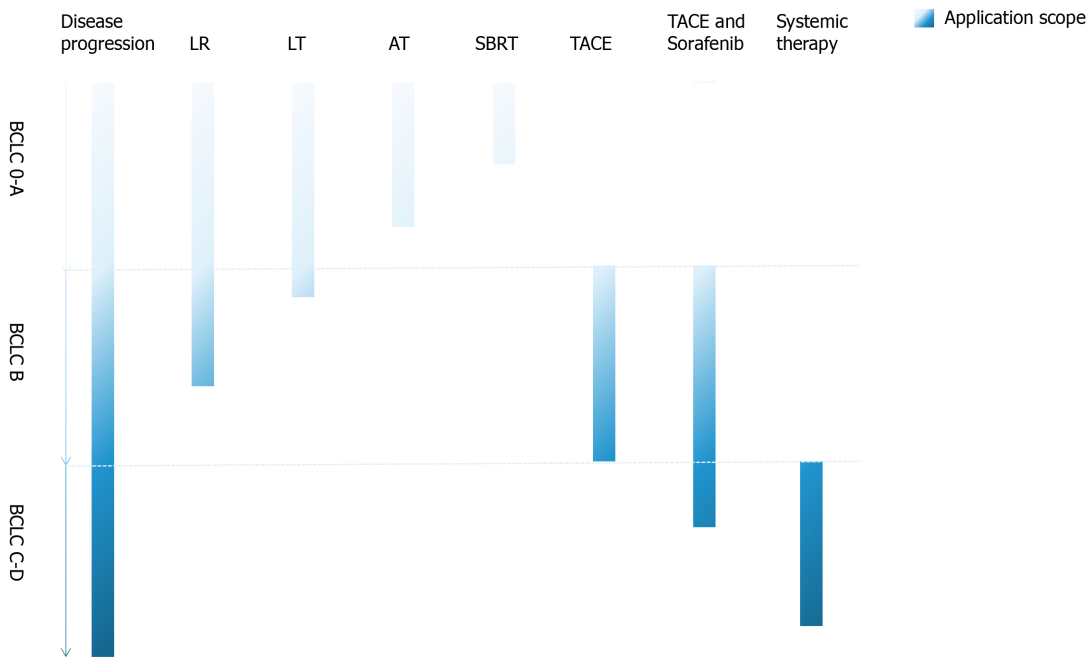
Figure 2 Therapeutic strategies for different stage hepatocellular carcinoma. Regarding therapeutic strategies, patients with early-stage hepatocellular carcinoma should be elevated for curative treatments including hepatectomy, liver transplantation (LT), or ablation depending on the patient performance status, underlying liver function, as well as disease-specific characteristics. AT: Ablation therapy; LR: Liver resection; SBRT: Stereotactic body radiation therapy; TACE: Transcatheter arterial chemoembolization.

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