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HEPATIC CANCER (N PARIKH, SECTION EDITOR)

Liquid Biopsy for Hepatocellular Carcinoma

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Abstract

Purpose of Review Clinically available biomarkers for hepatocellular carcinoma (HCC) early diagnosis and prognostication have limited utility. Further lack of routine biopsy in hepatocellular carcinoma limits the availability of molecular information to guide drug development. Recent studies investigating liquid biopsy using circulating tumor cells (CTCs) and cell-free deoxyribonucleic acid (cfDNA) have yielded promising data that could address both of these limitations.

Recent Findings For early HCC diagnosis, CTCs have modest sensitivity but high specificity. CfDNA methylation scores have shown high sensitivity and specificity in two large phase II studies. Presence of CTCs has been associated with poorer prognosis in numerous studies, particularly increased cancer recurrence following curative therapy, while the literature on cfDNA and prognosis is less robust.

Summary Liquid biopsy using CTCs and cfDNA has shown promise in prognostication and early diagnosis in HCC. Further robust validation of this liquid biopsy is required for routine clinical use.

Keywords Circulating tumor cell · Cell-free DNA · Early diagnosis · Biomarker

Abbreviations

AFP	Alpha-fetoprotein
cfDNA	Cell-free deoxyribonucleic acid
CTC	Circulating tumor cell
EMT	Epithelial-mesenchymal transition
HCC	Hepatocellular carcinomas

Introduction

Hepatocellular carcinoma (HCC) is the most common primary liver cancer and a leading cause of cancer death [1]. HCC incidence in the USA is rising, primarily due to increasing non-alcoholic fatty liver disease prevalence and peaking hepatitis C-related complications, and is one of few malignancies in the USA whose attributable mortality is increasing [2, 3]. HCC carries a poor prognosis with median survival under two years and is one of few malignancies in the USA. Several major challenges in HCC care exist. First, most patients are diagnosed at an advanced stage, partly because the current surveillance method of ultrasound and alpha-fetoprotein (AFP) has limited sensitivity and poor compliance [4]. Second, in patients with advanced-stage disease, currently available systemic therapy is ineffective, with objective response rates of only 10–20% [5, 6].

One factor limiting advances in HCC care and development of targeted systemic therapies is lack of biopsy tissue: HCC can be diagnosed and treated based on imaging alone and biopsy is not generally obtained [7]. Partly due to this, our understanding of HCC cancer biology lags behind that of other cancer types [8]. "Liquid biopsy," i.e., analysis of circulating tumor-derived molecules, may address this limitation by offering molecular insights into established cancer and serving as an early diagnostic biomarker for surveillance.

Circulating tumor cells (CTCs) and cell-free deoxyribonucleic acid (cfDNA) for early cancer detection, prognostication, and guiding choice of therapy will be the focus of this review. CTCs are believed to represent an intermediate stage between localized disease and distant metastasis and have been detected in virtually all major solid tumors [9]. CfDNA is released into the circulation by death of and secretion from cells and can be quantified, characterized for integrity, and sequenced to detect mutations, methylation, and insertion-deletions [10].

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(The term "circulating tumor DNA" is frequently used in the literature and refers to the subset of cfDNA that originates from tumors.) Non-coding ribonucleic acid can be detected in serum of patients with HCC or chronic liver disease, but we will focus on CTCs and cfDNA these non-coding RNA have been recently reviewed [11, 12].

Early Diagnosis

Circulating Tumor Cells

CTCs can be isolated from whole blood using a number of methods. The most commonly used and only Food and Drugs Administration–cleared method is CellSearch, which detects cells with an epithelial phenotype, i.e., cells which express EPCAM and cytokeratins [9]. Other methods exploit the fact that CTCs are typically larger than white blood cells: for example, CanPatrol utilizes size- and shape-based filtration, generally followed by fluoresecent labeling for cell-surface markers of interest [13], while other methods use microfluidics [14].

Several studies have investigated the utility of CTC in early diagnosis of HCC. Overall, CTC detection has moderate sensitivity (60–70%) and high specificity (> 70%) for distinguishing HCC from chronic liver disease or healthy controls [15]. One study of 296 HCC and 39 benign liver disease patients found a sensitivity of 65% using CanPatrol, a method based on filtration followed by fluoresecent labeling [16]. Studies using the commercially available CellSearch system have generally had lower sensitivities ranging from 31 to 68% [17, 18••].

High-throughput genetic analysis of CTCs or liver tissue can be used to develop predictive genetic scores for HCC [19•]. One study performed whole transcriptomic sequencing of circulating cells with epithelial phenotypes in patients with HCC vs. non-malignant chronic liver disease and found that cells from HCC patients had different transcriptomic profiles [20•]. They then created a score based on expression of specific genes to distinguish between HCC and chronic liver disease controls. Another study identified liver-specific transcripts and developed a serum risk score based on expression of these transcripts to distinguish between HCC and non-HCC controls [21]. While these genetic risk scores have not been externally validated, they may have greater sensitivity and specificity than those of CTCs alone.

Other attempts to develop more sensitive surrogate markers for CTCs have used targeted sequencing of genes such as *EPCAM*, whose corresponding protein has been used for CTC detection, or *AFP*, the protein product of which is clinically used as a noninvasive marker for HCC. One study of *EPCAM* mRNA found that *EPCAM* had lower sensitivity than AFP protein for HCC detection overall [22]. In two studies using *AFP* mRNA as a marker for HCC, sensitivity ranged from 54 to 100% depending on cancer stage, but specificity was modest at 56–86% [23, 24]. Use of multiple mRNAs may result in superior test characteristics: one such study used a combination of *EPCAM*, *CD133*, *CD90*, and *CK19* mRNA expression and reported sensitivity 73–82% and specificity > 90% (vs. chronic liver disease patients), with similar performance in early stage and AFP-negative HCC [25•]. Additional prospective studies on sequencing of CTC-associated genes are needed.

Cell-Free DNA

Various properties of cfDNA have been evaluated for early HCC diagnosis (Table 1). Early studies in this field investigated mutations in *TP53* [43, 47] or methylation of *P16* [39, 41], which generally had high specificity but low sensitivity around 26–55% depending on the control group. Similarly, other hotspot mutations in *TERT* promoter and *CTNNB1* are present in < 50% of patients with HCC [48, 50]. More recently, some studies have investigated total cfDNA amount or cfDNA integrity (defined as ratio of short- vs. long-circulating DNA strands), which generally have higher sensitivity around 70–80% and 80–90%, respectively [28–30, 34].

More recently, there has been interest in cfDNA methylation scores, which have yielded substantially higher sensitivity and specificity. One recent study from the USA identified a panel of six cfDNA differentially methylation regions that was tested in a cohort of 21 patients with HCC and 30 with cirrhosis, then validated in another with 95 HCC patients, 51 cirrhosis patients, and 98 healthy controls [51•]. On cross-validation, the sensitivity was 85% and specificity 91%, with sensitivity 75% for BCLC stage 0 and 93% for BCLC stage A HCC. Another larger study from China identified a set of ten cfDNA methylation markers on a training set of 715 HCC and 560 healthy controls, then validated these markers on 383 HCC patients and 275 controls [52••]. The authors reported sensitivity 83–86% and specificity 91–94% for distinguishing HCC from healthy controls. The authors also reported that this methylation score could also distinguish between HCC and non-malignant chronic liver disease (viral hepatitis and fatty liver), but sensitivity/specificity and numbers of the chronic liver disease patients were not reported.

Limitations

The existing literature on early HCC diagnosis using CTCs and cfDNA suffer from several major limitations. First, many of the studies detailed above, including the two large studies on methylation scores, included healthy individuals in their control arms, which could have resulted in inflated sensitivity/specificity estimates and is not a clinically relevant comparison. Also, some studies included patients with

Table 1 Selected studies on use of cell-free DNA for hepatocellular carcinoma diagnosis

Author	Year	Reference	Property	Country	Number of patients			HCC vs. CLD		HCC vs. healthy	
					HCC	CLD	Healthy	Sens	Spec	Sens	Spec
Chen	2012	[26]	Amount	China	80	80	50	72.5%	68.8%	86.3%	80.0%
Chen	2013	[27]		China	39		45			56.4%	95.6%
El-Shazly	2009	[28]		Egypt		25		72.0%	68.0%		
Huang	2012	[29]		China	72	37	41	69.4%	78.4%	90.3%	90.2%
Iizuka	2006	[30]		Japan		30		69.2%	93.3%		
Piciocchi	2013	[31]		Italy		76		90.9%	43.4%		
Ren	2006	[32]		China	79		20			51.9%	95.0%
Yan	2018	[33]		China		62		62.5%	93.5%		
Chen	2012	[26]	Integrity	China	80	80	50	91.3%	85.0%	86.3%	78.0%
El-Shazly	2009	[28]		Egypt		25		88.0%	92.0%		
Huang	2016	[34]		China	53	15	22	60.4%	80.0%	90.6%	90.9%
Chang	2008	[35]	*P16* methylation	China		16		19.2%	100.0%		
Chu	2004	[36]		Korea		23		47.8%	82.6%		
Tan	2007	[37]		Singapore	8		10			100.0%	100.0%
Wong	1999	[38]		Hong Kong	22	38	10	50.0%	100.0%	50.0%	100.0%
Wong	2000	[39]		Hong Kong	25	35	20	60.0%	100.0%	60.0%	100.0%
Wong	2000	[40]		Hong Kong	40	38	10	52.5%	100.0%	52.5%	100.0%
Wong	2003	[41]		Hong Kong	29	20	15	79.3%	100.0%	79.3%	100.0%
Zhang	2006	[42]		Taiwan	40		1			30.0%	100.0%
Huang	2003	[43]	*TP53* mutation	China	25	20	30	40.0%	80.0%	40.0%	93.3%
Igetei	2008	[44]		Nigeria	85		77			7.1%	100.0%
Jackson	2001	[45]		USA, China	15		18			46.7%	100.0%
Jackson	2003	[46]		USA, China	20		10			50.0%	100.0%
Kirk	2005	[47]		Gambia	186	98	348	39.8%	84.7%	39.8%	96.6%
Liao	2016	[48]		China	41		10			4.9%	100.0%
Marchio	2018	[49]		Cameroon, Central African Republic	149	164	49	24.8%	94.5%	24.8%	93.9%

HCC, hepatocellular carcinoma; *CLD*, chronic liver disease; *Sens*, sensitivity; *Spec*, specificity

advanced-stage HCC, but generally the more clinically relevant question is comparing early stage HCC to chronic liver disease controls, in order to achieve early detection. Most cfDNA studies utilized post hoc cutoffs of mRNA expression and cross-cohort validity remain to be determined; further, cross-ancestry validity of cfDNA scores remains to be determined.

Prognosis

Circulating Tumor Cells

Table 2 is a partial list of studies on the prognostic significance of CTCs in HCC. The literature on CTCs in prognosis in HCC is most robust in patients undergoing partial hepatectomy with curative intent [15]. In this population, unadjusted hazard ratio for recurrence after resection was 2.7 (95% confidence interval 2.1–3.4). Overall survival as an outcome has been less consistently reported in patients undergoing resection, but presence of CTCs is usually associated with poorer overall survival as well [17, 57].

The utility of CTCs for prognostication is less clear in patients with intermediate- or advanced-stage disease. In patients receiving locoregional therapy, presence of CTCs has been associated with progression [22] and poorer overall survival [63, 68], but other studies showed no significant associations [69, 70]. Notably, the sizes of the cohorts including intermediate- and advanced-stage HCC are far smaller than those of the resection studies. Among patients receiving systemic therapy, there are very limited data on the association between CTCs and prognosis. Two small studies in patients with advanced-stage disease, one of the patients receiving sorafenib and temsirolimus and the other of patients not on systemic therapy at time of enrollment, showed no difference in overall survival based on presence or absence of CTCs [71, 72]. Another study showed a significant association between CTC presence and progression-free survival, but the definition

Table 2 Selected studies on use of circulating tumor cells for hepatocellular carcinoma prognosis

Author	Year	Ref	CTC type	Country	N	Treatment type/stage	Outcome	Hazard ratio (95% CI)	UV HR?	Covariates
Court	2018	[53]	E	USA	80	Mixed	OS	2.21 (1.38–3.52)	Y	
					30	Metastatic disease	PFS	1.81 (1.02–3.22)	Y	
					23	Curative therapy	TTR	3.14 (1.5–6.57)	Y	
Fan	2011	[54]	O	Hong Kong	82	Surgery	OS	4.74 (1.71–13.12)	N	Tumor size, tumor count, TNM stage, blood transfusion
					82	Surgery	RFS	4.17 (2.14–8.13)		
					82	Surgery	Recurrence	6.26 (2.18–18.03)		
Guo	2014	[22]	E	China	122	Surgery	Recurrence	2.71 (1.52–4.86)	Y	
					56	TACE	Progression	3.75 (1.41–9.97)	Y	
					44	Radiotherapy	Progression	5.07 (1.39–18.47)	Y	
Guo	2018	[25•]	O	China	130	Surgery	Recurrence	2.46 (1.49–4.09)	Y	
					195	Surgery	Recurrence	2.37 (1.11–5.07)	Y	
Lai	2016	[55]	O	China	75	Surgery	DFS	0.42 (0.18–0.95)	Y	
			O		75	Surgery	DFS	2.71 (1.12–6.56)	Y	
Li	2016	[56]	O	China	59	Sorafenib	PFS	9.39 (3.24–27.19)	N	Child-Pugh score, TNM stage
Liu	2013	[57]	O	China	60	Surgery	DFS	7.15 (2.99–17.09)	N	Tumor size, tumor count, alpha-fetoprotein, portal vein thrombus, ascites
					60	Surgery	OS	2.28 (0.95–7.82)	N	Portal vein thrombus, ascites, prealbumin
Nel	2013	[58]	O	Germany	11	Mixed	TTP	0.18 (0.01–2.75)	Y	
			O		11	Mixed	TTP	0.19 (0.01–2.75)	Y	
Ogle	2016	[59]	MI	UK	69	Mixed	OS	2.34 (1–5.42)	N	Tumor size, portal vein thrombus, metastases
Ou	2018	[60]	E	China	165	Surgery	RFS	1.45 (0.67–3.13)	Y	
			O		165	Surgery	RFS	4.55 (2.2–9.38)	Y	
			O		165	Surgery	RFS	2.37 (0.81–69.37)	Y	
Qi	2018	[61]	MI	China	112	Surgery	Recurrence	1.04 (1.03–1.05)	Y	
Schulze	2017	[62]	E	Germany	57	Surgery	Recurrence	2.3 (1.1–4.8)	Y	
Shen	2018	[63]	E	China	97	Locoregional	Death	4.16 (2.04–8.49)	Y	
					97	Locoregional	Death	1.7 (0.88–3.25)	Y	
Sun	2013	[64]	E	China	123	Surgery	Recurrence	5.37 (2.92–9.85)	Y	
Sun	2018	[18••]	E	China	73	Surgery	Intrahepatic recurrence	7.87 (2.87–21.59)	Y	
Von Feld-on	2017	[65]	E	Germany	57	Surgery	Recurrence	2.3 (1–5.2)	Y	
Wang	2018	[66]	MI	China	62	Surgery	Recurrence	2.95 (1.18–7.35)	Y	
Xue	2018	[67]	MI	China	30	Transplant	RFS	5.14 (1.53–17.3)	Y	
			E		30	Transplant	RFS	0.54 (0.12–2.49)	Y	
Yu	2018	[17]	E	China	139	Surgery	DFS	0.53 (0.41–0.68)	Y	
					139	Surgery	OS	0.48 (0.36–0.66)	Y	
Zhou	2016	[68]	E	China	49	Surgery	Recurrence	6.58 (2.06–21.05)	Y	
					19	Locoregional	OS	5.02 (1.26–19.93)		

Ref, reference number; *CTC*, circulating tumor cell; *CI*, confidence interval; *UV HR*, univariate hazard ratio; *HCC*, hepatocellular carcinoma; *CLD*, chronic liver disease; *E*, CTC detection based on epithelial markers; *MI*, marker-independent CTC detection; *O*, other; *TACE*, transarterial chemoembolization; *OS*, overall survival; *RFS*, recurrence-free survival; *PFS*, progression-free survival; *TTP*, time to progression; *TNM*, tumor-node-metastasis

of CTCs used here (phosphorylated ERK or Akt) has not been separately validated in HCC [56].

CTCs may be in a sense more relevant in patients with early stage disease as they indicate micrometastatic disease that may not be clinically apparent. In contrast, patients with advanced-stage HCC have by definition overt portal vein involvement or extrahepatic metastasis. However, further studies using larger cohorts of patients with intermediate- or advanced-stage disease will be required to better evaluate the significance of CTCs in this population.

CTCs can have different phenotypes, namely an epithelial phenotype, a mesenchymal phenotype, or a mixed phenotype; the epithelial-mesenchymal transition (EMT) is believed to be an important step in carcinogenesis that facilitates invasion into the circulation and, thus, metastasis [73]. As with other cancer types, in HCC primary tumors, expression of EMT genes is associated with poorer prognosis [74]. Likewise, CTCs with mesenchymal phenotypes appear to be associated with a poorer prognosis in HCC. CTC expression of the EMT proteins Slug, Snail, Twist, ZEB1, or Vimentin has been associated with the presence of tumor thrombus and metastatic disease [18••, 75]. A more recent study found that in patients undergoing curative resection, post-operative presence of mesenchymal CTCs has been associated with increased risk of early HCC recurrence, while presence of epithelial or mixed CTCs was not [76]. Whole transcriptome sequencing of CTCs has also been performed in limited studies; however, whether transcriptomic analysis of HCC CTCs yields information beyond that of targeted sequencing of candidate genes is not known [19•].

Location of CTCs may also have clinical significance. One study measured CTC counts in different vascular spaces of patients undergoing curative resection for HCC, including peripheral veins and arteries, portal vein, hepatic vein, and infrahepatic inferior vena cava [25•]. As might be predicted, CTC counts were highest in the hepatic vein, then in the peripheral vein, then lowest from the other vascular territories. Further, these vascular spaces had different clinical significance. Patients who subsequently developed intrahepatic recurrence had higher numbers of CTCs in the systemic circulation but similar numbers of CTCs in the hepatic circulation, compared with patients without intrahepatic recurrence. In contrast, those who developed lung metastasis had more CTCs in the hepatic vein but similar numbers of systemic circulation CTCs. While it is not practical to routinely sample the hepatic vein in all patients, it may be useful for risk stratification in patients undergoing resection or transplantation.

Circulating tumor clusters consisting of CTCs and, possibly, white blood cells and stromal cells may have special clinical significance. Several recent studies in breast cancer showed that circulating tumor clusters are more tumorigenic, perhaps because clusters are enriched for cancer stem cells whereas single circulating tumor cells are not [77]. Circulating tumor clusters are not well-studied in HCC, but one study suggested that they may portend an even poorer prognosis than single CTCs alone [18••].

Cell-Free DNA

Studies on cfDNA have evaluated various properties for their effect on prognosis. However, these studies are typically less standardized in terms of tumor stage and treatment type than studies on CTCs, and there is insufficient evidence to comment on differential utility of cfDNA in early stage vs. advanced-stage disease. While some studies on total cfDNA amount showed poorer overall survival and earlier time to progression with higher cfDNA amounts [31, 78], other studies across a range of HCC stages found no association between cfDNA amount and overall survival [28, 29]. Timing of cfDNA collection may be significant: one study in patients receiving radiotherapy found that while pre-therapy cfDNA amount correlated with tumor size, post-therapy cfDNA amount was more prognostically significant and was associated with intrahepatic recurrence [79]. CfDNA integrity has not been well-studied in the setting of prognosis but may be associated with poorer overall survival [28]. Similarly, mutations in or methylation of candidate genes have been variably associated with poorer overall survival [80] or recurrence [40, 48], and requires further characterization.

As with early diagnosis, cfDNA methylation scores may have greater predictive power than individual hotspot mutations or cfDNA amount/integrity. One large study found that the same methylation score used for early diagnosis was associated with poorer survival in both derivation ($N = 680$) and validation ($N = 369$) cohorts, with primarily advanced-stage HCC (64% TNM stage III/IV) [52••]. This score added prognostic information beyond that of TNM stage alone. Another study found that having ≥ 3 of 6 methylation markers in *RASSF1A*, *CCND2*, *CFTR*, *SPINT2*, *SRD5A2*, and/or *BASP1* was associated with poorer adjusted disease-free survival and overall survival, in a mix of early and advanced-stage HCC (54% TNM stage III/IV) [81].

Limitations

Several limitations exist in the literature on liquid biopsy in for HCC prognostication. First, outcomes are inconsistently reported: usually studies in patients receiving curative therapy report recurrence or recurrence-free survival while those in patients receiving non-curative therapy report progression or progression-free survival, and overall survival is frequently not mentioned at all. In addition, some studies report hazard ratios while others describe mean/median overall survival, progression-free survival, etc. More consistent reporting will facilitate an improved understanding of the use of CTCs for predicting prognosis in HCC. Finally, cfDNA studies are

prone to ad hoc cutoffs in amount, integrity, or methylation proportion.

Liquid Biopsy to Guide Therapy

In several cancer types, genetic data are used to guide systemic therapy decisions. For example, erlotinib and alectinib are approved for lung adenocarcinoma with selected *EGFR* mutations and *EML4-ALK* fusions, respectively, while pembrolizumab is approved as second-line therapy for microsatellite instability-high or mismatch repair-deficient cancer regardless of primary site [82, 83]. In HCC, biomarkers to guide treatment in the setting of systemic disease are much more limited, and no therapies are approved for use specifically in tumors with specific genetic alterations. The SHARP study identified no biomarker as predictive of treatment response to sorafenib [84], and neither AFP nor c-Met is associated with response to regorafenib treatment [85]. Until recently, AFP was the only biomarker associated with response to certain treatments in HCC: ramucirumab improved overall survival in advanced-stage HCC only in patients with AFP concentration > 400 ng/mL [86], and survival benefit to cabozantinib was greater in HCC patients with serum AFP ≥ 200 ng/mL [87]. A recent study identified a set of microRNAs and plasma proteins associated with response to regorafenib, though this requires further validation [88]. No studies have identified biomarkers predicting response to checkpoint inhibitor therapy, which are currently approved as second-line agents in patients with unresectable HCC.

In order for liquid biopsy to be useful for guiding clinical decision-making, it should ideally reflect the biology of the primary tumor. In HCC, genetic alterations in cfDNA are inconsistently concordant with those of the primary tumor. In one study of paired tumor-plasma samples, 89% of patients with *TP53* R249S mutations in the primary tumor also had them in cfDNA [89], but in another study, only 69% of patients with *GSTP1* promoter hypermethylation in the primary tumor had this in cfDNA [90]. Several studies have systematically sequenced selected cancer-related genes in both cfDNA and primary tumors: while cfDNA mutations are typically detected in primary tumors, the sensitivity of cfDNA for primary tumor mutations is widely variable from 30 to > 90% and may vary based on the specific gene [91, 92]. It is not known whether HCC CTC mutations correlate with those of the primary tumor as to our knowledge DNA sequencing in HCC CTCs has not been reported. However, studies in prostate cancer [93] and multiple myeloma [94] suggest that mutations are usually concordant between CTCs and primary tumors. It is difficult to determine whether transcriptomes are concordant between primary tumor and CTCs due to tumor heterogeneity and small numbers of CTCs, but in prostate cancer, CTCs more closely resemble their corresponding primary tumor than primary tumors from other individuals [93].

Liquid biopsy could hypothetically guide therapy in a few ways. One would be to use liquid biopsy to guide choice of initial therapy. Using cfDNA to identify specific mutations is unlikely, at present, to be an adequate tool to achieve this, since most patients with HCC do not have targetable mutations [95]. Other properties of cfDNA have been explored as well: for instance, genomic instability and total amount have been associated with poorer response to sorafenib [96]. CTCs may provide an alternate method of identifying response to therapy: they can be characterized by transcriptomic analysis or proteomics, which would potentially allow for expression profiles (rather than mutations only) that are associated with response to one drug or another. While whole transcriptome sequencing of HCC CTCs has been performed [19•, 20•], whether it has implications for treatment response remains to be determined.

Another potential application of liquid biopsy is to monitor patients on therapy and determine whether to continue treatment or switch to another option. RNA-based scores (as an approximation of CTCs) have been used to monitor response to treatment in HCC [21] but to our knowledge, there are no published studies showing that liquid biopsy for monitoring is superior or comparable to imaging studies. Literature in other cancer types may provide a roadmap to liquid biopsy-guided HCC treatment. In prostate cancer, cfDNA mutational profiles may predict development of enzalutamide resistance [97]. In breast cancer, cfDNA correlates with tumor burden and may be an early predictor of disease progression or recurrence [98]. Monitoring may not result in clinically relevant improvement in outcomes though. One study of 319 patients with metastatic breast cancer and detectable CTCs at baseline who were receiving standard first-line chemotherapy followed CTC counts longitudinally; patients with persistently increased CTCs were randomized to either change to an alternative chemotherapy regimen or continue current treatment, with the idea that persistently increased CTC count indicated treatment failure [99]. This was a negative study: there was no change in overall survival in the patients who underwent CTC-guided change in therapy compared with those who did not. Prospective studies on this topic in HCC are required to further clarify any potential role of liquid biopsy for monitoring.

Currently, no major professional societies endorse use of CTCs or cfDNA to guide treatment or for monitoring across all cancers. We agree that it would be premature to use liquid biopsy as reviewed in this article to guide therapy at this time. However, studies on CTCs and cfDNA in other cancer types suggest that in the future it may be possible to apply these methods to HCC as well.

Conclusions

Liquid biopsy has the potential to address two of the major limitations in clinical HCC management, namely inadequate HCC surveillance tools and providing molecular data, that will, hopefully, one day help guide choice of systemic therapy in HCC. We believe that liquid biopsy using CTCs and cfDNA is promising in early diagnosis and prognostication of HCC. CfDNA has shown the most potential for early diagnosis, especially if polygenic scores are used; in contrast, CTCs have only modest sensitivity for early HCC detection. In contrast, the data for CTCs and HCC prognosis are more robust, and CTCs also offer the opportunity to characterize the cancer transcriptome noninvasively. However, the field is still immature and there are inadequate data to recommend using liquid biopsy to determine choice of initial treatment or for monitoring on treatment. Further research is required to further evaluate potential clinical applications of liquid biopsy in HCC.

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• Of importance
•• Of major importance

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