

Prognostic Significance of Liquid Biopsy-Detected Genetic Alterations in Hepatocellular Carcinoma

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Background: Hepatocellular carcinoma (HCC) is a leading cause of cancer-related mortality worldwide

and is increasingly diagnosed in younger populations. Conventional biopsy techniques can be invasive and may not accurately capture tumor heterogeneity. Liquid biopsy, analyzing circulating tumor DNA (ctDNA), offers a minimally invasive and dynamic alternative for detecting genetic alterations critical to early diagnosis and personalized treatment strategies.

Methods: We analyzed serum-derived ctDNA from 20 HCC patients to identify genetic variants using next-generation sequencing (NGS). Mutations in key oncogenes and tumor suppressor genes (eg, KIT, FGFR1, FGFR3, EGFR, BRAF, FBXW7) were evaluated for their association with clinical outcomes, including tumor size, metastasis, and overall survival. Statistical analyses were performed using SPSS (v.30), with survival curves assessed via the Kaplan-Meier method.

Results: Of the 20 patients (mean age 64.8±13.1 years), 35% had detectable ctDNA mutations. The most frequently observed alterations were in KIT (28.6% of ctDNA-positive patients), followed by FGFR1, FGFR3, EGFR, BRAF, and FBXW7. Patients harboring FGFR1 and FGFR3 mutations exhibited the poorest survival (3 and 7 months, respectively). Conversely, one patient with a BRAF mutation showed prolonged survival (60 months), and KIT mutations were linked to comparatively better outcomes. Overall, ctDNA-positive patients demonstrated shorter mean survival (22.5 months) than ctDNA-negative patients (35.7 months).

Conclusion: Liquid biopsy-detected genetic alterations correlate with clinical outcomes in HCC, underscoring the prognostic value of ctDNA analysis. Mutations in FGFR1 and FGFR3 were associated with aggressive disease, suggesting these pathways as potential therapeutic targets. Integrating liquid biopsy with other diagnostic modalities may enhance personalized management and improve prognosis for patients with HCC.

Plain Language Summary: Hepatocellular carcinoma (HCC) is a serious type of liver cancer that affects thousands of people each year. Detecting and treating it early can be difficult. Traditional tests often require taking tissue samples, which can be uncomfortable and risky for the patient.

Our study looked at a blood-based test called a “liquid biopsy”. Liquid biopsy tests for tiny fragments of cancer DNA (called circulating tumor DNA) in the bloodstream. We wanted to see if certain changes in this DNA might predict whether a patient’s cancer could grow or spread.

We analyzed blood samples from 20 people with HCC. We found that some had specific genetic changes, especially in genes called FGFR1 and FGFR3. People with these changes had a shorter life expectancy compared to those without them. On the other hand, different genetic changes in the BRAF and KIT genes were linked to longer survival. These findings suggest that liquid biopsy could help doctors identify patients who are at higher risk and may need more aggressive treatment. It may also help in choosing targeted therapies to block specific genetic changes. Overall, using liquid biopsy could lead to better and more personalized ways of treating liver cancer, with fewer side effects than traditional methods.

Keywords: hepatocellular carcinoma, liquid biopsy, genetic alterations, next-generation sequencing, prognosis

Introduction

Hepatocellular carcinoma (HCC) is one of the most common and deadly forms of liver cancer, accounting for a significant portion of cancer-related deaths worldwide.¹ Even though the diagnosis of HCC peaks in 7th decade of life, according to the American Cancer Society's 2024 data, its incidence is increasing annually in patients younger than 50.² Despite advances in treatment, the prognosis for HCC remains poor, largely due to late-stage diagnosis and the high propensity for metastasis. Traditionally, diagnosis and monitoring of HCC have relied on imaging techniques and tissue biopsies, which can be invasive, costly, and sometimes inaccurate.¹

In recent years, liquid biopsy has emerged as a transformative tool for cancer detection and monitoring. This minimally invasive approach analyzes circulating tumor-derived components—including circulating tumor cells (CTCs), circulating tumor DNA (ctDNA), and extracellular vesicles—to provide real-time insights into tumor genetics, clonal evolution, and treatment resistance mechanisms. Unlike traditional biopsies, liquid biopsy allows for serial sampling, enabling dynamic assessment of tumor burden and molecular heterogeneity without procedural complications. One of the most significant advantages of liquid biopsy is its ability to provide real-time insights into the tumor's genetic profile without the need for repeated invasive procedures. This is especially valuable for cancers such as hepatocellular carcinoma (HCC), where the tumor's molecular landscape can evolve over time, influencing treatment response and prognosis. Liquid biopsy allows for longitudinal monitoring, enabling clinicians to track tumor progression, assess minimal residual disease (MRD), and detect early signs of recurrence or metastasis. For HCC, where early detection is critical yet challenging, liquid biopsy holds particular promise. Studies have demonstrated its utility in detecting actionable mutations (eg, TERT promoter, TP53), monitoring minimal residual disease, and predicting therapeutic response to systemic therapies such as tyrosine kinase inhibitors.^{3,4}

By offering a less invasive, yet highly informative diagnostic tool, liquid biopsy holds the potential to revolutionize the management of HCC. It enables early detection, continuous monitoring of treatment response, and the identification of minimal residual disease, thereby improving patient outcomes. Overall, the clinical utility of liquid biopsy in HCC and other cancers cannot be overstated. By providing a less invasive, more dynamic, and real-time method for monitoring disease progression, detecting early signs of recurrence, and guiding therapeutic decisions, liquid biopsy has the potential to significantly improve patient outcomes. Furthermore, its ability to monitor the tumor's evolving genetic profile makes it an indispensable tool in the age of precision medicine, where treatments are increasingly tailored to the unique characteristics of each patient's cancer. Moreover, the ability to perform serial sampling makes liquid biopsy particularly valuable in tracking tumor evolution and resistance mechanisms, paving the way for personalized treatment strategies.⁵

In this study, we aimed to present our liquid biopsy results and their correlation with clinical parameters in patients diagnosed with hepatocellular carcinoma. By analyzing the genetic mutations detected through liquid biopsy, we sought to evaluate their potential associations with clinical outcomes, including disease progression, treatment response, and overall prognosis.

Materials and Method

This prospective study enrolled patients diagnosed with hepatocellular carcinoma (HCC) at the Department of General Surgery, Çukurova University Faculty of Medicine, between January 2020 and December 2022. Inclusion criteria were: HCC confirmed by clinical, radiological or histopathological criteria; availability of complete liquid biopsy data; and written informed consent. Exclusion criteria included incomplete medical records, insufficient follow-up (<6 months), or missing genetic analysis results. Ethical approval was obtained from the Çukurova University Faculty of Medicine Ethics Committee for Non-Invasive Clinical Research (Decision No. 12; February 14, 2020).

Demographic data of the patients, including age, gender, and comorbidities (eg, diabetes, hypertension, chronic liver diseases), were collected. Laboratory parameters evaluated included alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin, direct bilirubin, albumin, international normalized ratio (INR), creatinine, gamma-glutamyl transferase (GGT), alkaline phosphatase (ALP), alpha-fetoprotein (AFP), Ca 19.9, and carcinoembryonic antigen (CEA). Tumor characteristics (solitary/multiple, diameter, location, PET-CT avidity, metastasis sites) and survival data (overall survival from diagnosis to death/last follow-up) were extracted from institutional databases.

For liquid biopsy analysis, circulating tumor DNA (ctDNA) was isolated from serum samples. Next-generation sequencing (NGS) was performed using the GeneReader NGS System to analyze the following genes: *AKT1*, *ERBB2*, *FGFR2*, *KIT*, *NRAS*, *ALK*, *ERBB3*, *FGFR3*, *KRAS*, *PDGFRA*, *BRAF*, *ERBB4*, *FLT3*, *MAP2K1*, *PIK3CA*, *CTNNB1*, *ESR1*, *GNA11*, *MAP2K2*, *RAF1*, *DDR2*, *FBXW7*, *GNAQ*, *MET*, *SMAD4*, *EGFR*, *FGFR1*, *HRAS*, *NOTCH1*, and *STK11*. Additionally, amplifications of the following genes were assessed: *ALK*, *BRAF*, *EGFR*, *ERBB2*, *FGFR1*, *FGFR2*, *FLT3*, *KIT*, *KRAS*, *MAP2K1*, *MET*, and *PIK3CA*. Genetic data were compared against various databases, including the Human Gene Mutation Database (HGMD), Catalogue Of Somatic Mutations In Cancer (COSMIC), 1000 Genomes Project, and the Ingenuity Knowledge Base. Bioinformatics analyses were performed using QIAGEN Ingenuity Variant Analysis (IVA) and QIAGEN Clinical Insight (QCI) tools to interpret the clinical significance of the identified genetic variants.

Statistical analysis were performed using SPSS v.30 (IBM Corp., Armonk, NY). The normality of continuous variables was assessed using the Shapiro–Wilk test. Continuous variables with normal distribution were expressed as mean $\pm$ standard deviation (SD), while non-normally distributed variables were expressed as median (interquartile range, IQR). Comparisons between normally distributed continuous variables were conducted using independent samples *t*-tests, while the Mann–Whitney *U*-test was used for non-normally distributed variables. Categorical variables were presented as frequencies and percentages, and differences between groups were analyzed using the chi-square test. Survival analyses were performed using the Kaplan–Meier method, and comparisons between survival curves were evaluated with the Log rank test. A *p*-value of <0.05 was considered statistically significant for all analyses.

Results

A total of 20 patients diagnosed with hepatocellular carcinoma (HCC) were included in the study. The cohort consisted of 70% male patients and 30% female patients, with a mean age of 64.8 ± 13.1 years. Genetic mutations were identified through liquid biopsy in 35% of the patients ($n=7$), designated as the liquid biopsy positive (LB+) group. Detailed demographic data are presented in Table 1.

The most frequently detected genetic mutation was *KIT*, observed in 28.6% of LB+ patients, with two distinct mutations identified: c.1621A>C (p.M541L) and c.120_123delTCCA (p.H40fs*6). Additional mutations were identified in the following genes: *FGFR1*, *FGFR3*, *EGFR*, *BRAF*, and *FBXW7*. Table 2 provides a summary of the identified genetic mutations and their respective frequencies.

Laboratory values for the patients, categorized by the presence or absence of genetic mutations, are presented in Table 3. No statistically significant differences in laboratory parameters were observed between the LB+ and LB− groups.

The clinical characteristics of the study participants are summarized in Table 4. Analysis of clinical data revealed no statistically significant differences between the groups with respect to tumor number (solitary or multiple), tumor size, location, or the presence of metastasis. However, certain trends were noted. Specifically, patients with *EGFR* and *KIT*

Table 1 Demographical Data of the Patients

Parameter	LB(-) (n=13)	LB(+) (n=7)	Total (n=20)	p value
Age	62.3 $\pm$ 13.0	69.4 $\pm$ 13.1	64.8 $\pm$ 13.1	0.258
Gender				
Male	84.6%(11)	42.9%(3)	70.0%(14)	0.05
Female	15.4%(2)	57.1%(4)	30.0%(6)	
Comorbidities				
Hypertension	30.8%(4)	57.1%(4)	40%(8)	0.251
Diabetes Mellitus	38.5%(5)	14.3%(1)	30%(6)	0.260
Cirrhosis	61.5%(8)	42.9%(3)	55.0%(11)	0.423
Coronary Artery Disease	23.1%(3)	14.3%(1)	20%(4)	0.639

Notes: Categorical data are presented as percent (count), while numerical data are presented as mean $\pm$ standard deviation.

Table 2 Genetical Mutations

Gene	Mutation	Percentage
KIT(n=2)	c.120_123delTCCA(p.H40fs*6). c.1621A>C(p.M541L)	28.6%
FGFR1(n=1)	c.396_398delTGA(p.D133del)	14.3%
FGFR3 (n=1)	c.1206delC(p.K403fs*29)	14.3%
EGFR(n=1)	c.1787>G(p596R)	14.3%
BRAF(n=1)	c.1324G>A(p.G442S)	14.3%
FBXW7(n=1)	c.1436G>T(p.R479L)	14.3%

Table 3 Laboratory Values

Parameter	LB (-) (n=13)	LB (+) (n=7)	P value
ALT	38.00 (25.00–58.00)	25.00 (18.50–35.00)	0.096
AST	63.00 (43.00–126.00)	49.00 (35.00–82.00)	0.579
Albumin	33.82±6.80	32.02±4.99	0.548
INR	1.22±0.16	1.27±0.32	0.637
Creatine	0.78±0.15	0.94±0.33	0.150
Amylase	64.06±19.9	66.56±20.5	0.794
GGT	94.00 (31.00–213.00)	68.00 (32.00–75.50)	0.405
ALP	133.00 (116.00–247.00)	125.00 (95.00–148.50)	0.475
AFP	21.07 (3.20–1230.90)	258.30 (86.10–7737.80)	0.438
CA19.9	51.40 (16.30–65.10)	25.70 (10.95–36.80)	0.233
CEA	2.74 (2.03–3.13)	2.97 (2.74–3.16)	0.579

Notes: Data that follows a normal distribution are presented as the mean ± standard deviation, while non-normally distributed data are presented as the median (interquartile range).

Table 4 Comparison of Clinical Data Between Groups

Parameter		LB (-) (n=13)	LB (+) (n=7)	Total (n=20)	P value
Tumor Diameter(cm)	1-3	0	28.6% (2)	10% (2)	0.116
	3-5	30.8%(4)	14.3%(1)	25%(5)	
	>5	69.2%(9)	57.1%(4)	65%(13)	
Number of masses	Single	76.9%(10)	71.4%(5)	75.0%(15)	0.541
	Multiple	23.1%(3)	28.6%(2)	25.0%(5)	
Metastasis	Yes	38.5%(5)	42.9%(3)	40.0%(8)	0.848
	No	61.5%(8)	57.1%(4)	60.0%(12)	
Localization of tumor	Left	15.4%(2)	28.6%(2)	20.0%(4)	0.354
	Right	61.5%(8)	71.4%(5)	65.0%(13)	
	Bilobar	23.1%(3)	0	15.0%(3)	
SUVMax		7.88(5.7–12.6)	8.16(7.5–8.8)	7.8(7.3–8.8)	0.99

Notes: Categorical data are presented as percent (count), while numerical data are presented as median (interquartile range).

mutations (c.120_123delTCCA [p.H40fs*6]) tended to have smaller tumors. Metastatic involvement was observed in patients harboring mutations in the *FBXW7*, *FGFR3*, and *KIT* (c.1621A>C [p.M541L]) genes.

Survival outcomes were also analyzed in relation to liquid biopsy results. Patients with negative liquid biopsy results (LB–) had a significantly longer mean survival of 35.7 months (95% CI: 18.7–52.7), compared to those with positive liquid biopsy results (LB+), who had a mean survival of 22.5 months (95% CI: 8.0–37.1) (Figure 1). Further analysis of the genetic mutations in LB+ patients revealed that one patient with a BRAF mutation remained alive with a follow-up period of 60 months. Among patients with KIT mutations, those with the c.120_123delTCCA (p.H40fs*6) mutation had

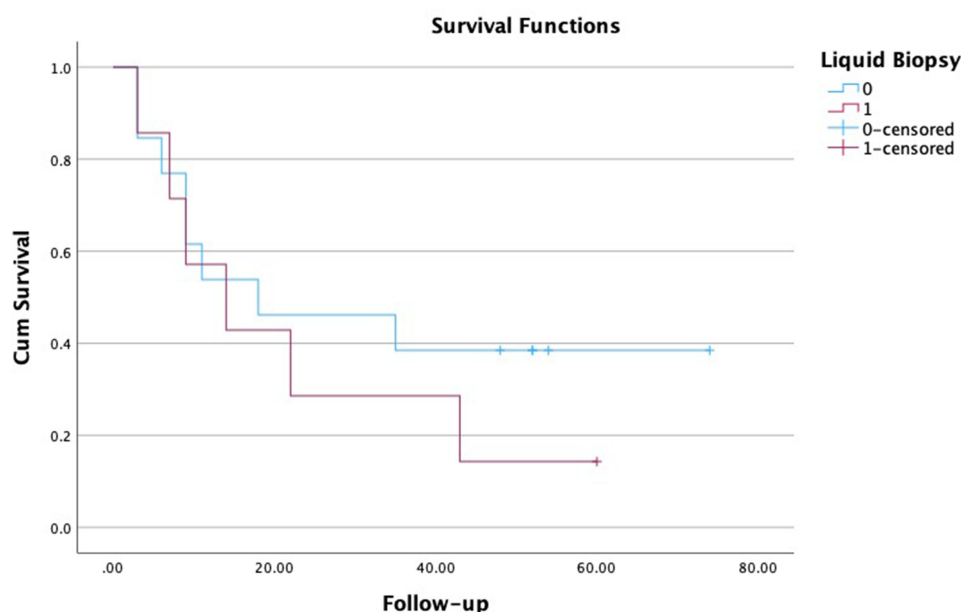


Figure 1 Survival analysis of LB(+) and LB(-) groups.

a longer survival (43 months) compared to patients with the c.1621A>C (p.M541L) mutation, who had a survival of 22 months. The poorest prognosis was observed in patients with mutations in the FGFR gene: patients with FGFR1 mutations had a survival of only 3 months, and those with FGFR3 mutations had a survival of 7 months.

Discussion

Liquid biopsy has emerged as a promising non-invasive diagnostic tool in the management of hepatocellular carcinoma (HCC). Due to its minimally invasive nature, liquid biopsy offers significant advantages over traditional methods like tissue biopsies, which are often associated with procedural risks, costs, and limited accessibility. By utilizing cell-free DNA (cfDNA) analysis, liquid biopsy allows for the detection of genetic mutations, real-time monitoring of treatment responses, and tailoring of drug selection and dosage based on tumor-specific molecular profiles. This approach has demonstrated high sensitivity, which can be critical in the early detection of HCC and in assessing disease progression and therapeutic efficacy.⁶

In our study, mutations in the FGFR1 and FGFR3 genes were associated with poorer survival outcomes compared to other mutations. These findings are in line with the well-established role of the fibroblast growth factor (FGF) signaling pathway in cancer biology. The FGF family and its receptors (FGFRs) play pivotal roles in regulating tumor growth, angiogenesis, and metastasis. Abnormalities in the FGFR signaling pathway can lead to uncontrolled cell proliferation, migration, and survival, which are key processes in the initiation and progression of HCC.⁷⁻⁹ Moreover, aberrant FGFR signaling has been implicated in the development of resistance to therapy, particularly in cancers that are not responsive to traditional chemotherapy. Consequently, pan-FGFR inhibitors are being actively investigated in preclinical studies and Phase I clinical trials as potential therapeutic options for targeting these aberrant pathways in HCC and other cancers.^{10,11} The identification of mutations in these genes may not only serve as prognostic markers but also as targets for personalized therapeutic strategies.

The clinical impact of KIT mutations has been reported to vary heterogeneously across different cancer types and is influenced by the specific exon within the gene where the mutation occurs.¹² In our cohort, although the sample size was small, patients with KIT mutations demonstrated better survival outcomes compared to others, suggesting that KIT may play a prognostic role in HCC. In particular, mutations in the KIT gene have been associated with enhanced cell growth, survival, and motility, all of which contribute to cancer progression and metastasis. Recent research suggests that KIT mutations might influence tumor metastasis through alterations in tumor microenvironment interactions and by promoting epithelial-to-mesenchymal transition (EMT), a crucial step in the metastatic cascade. However, the mechanisms by

which KIT mutations promote metastasis in HCC remain to be fully elucidated, and larger studies are needed to confirm these associations and to explore the therapeutic potential of KIT-targeted therapies. As for the patient with a BRAF mutation, the classical V600 mutation was not observed.¹³ Previous studies have predominantly focused on immuno-histochemistry and expression patterns, making it challenging to establish a definitive relationship. This discrepancy suggests that BRAF mutations in HCC might involve different genetic alterations compared to those in other cancers. BRAF is a key player in the MAPK/ERK signaling pathway, which regulates cell growth, differentiation, and survival. Mutations in BRAF, particularly those outside the V600E hotspot, have been observed in various cancers and may have distinct roles in promoting tumorigenesis and metastasis. In HCC, non-V600 BRAF mutations have been less studied, and there is growing evidence that these alterations might contribute to tumor progression by activating alternative signaling pathways that bypass classical BRAF inhibition. Our study identified a patient with a non-V600 BRAF mutation, yet no direct association with progression or metastasis was observed, indicating that further research is needed to better understand the implications of these mutations in HCC.

Labgaa et al conducted a study on 8 patients to investigate specific mutations through both liquid biopsy and tissue biopsy methods. They analyzed 58 genes, including frequent HCC driver genes and druggable mutations, such as TERT promoter, TP53, NTRK3, and JAK1. Their findings demonstrated that ultra-deep sequencing of circulating-free DNA (cfDNA) effectively detects somatic mutations identified in tissue, confirming the tumoral origin of cfDNA and highlighting its potential as a minimally invasive tool in the genetic analysis of HCC.¹⁴ Similarly, Hirai et al conducted a study on 130 patients with advanced HCC to investigate the presence of TERT promoter mutations in ctDNA and their prognostic significance.¹⁵ They found that 54.6% of patients harbored TERT promoter mutations (49.2% with 124bp G>A and 7.8% with 146bp G>A mutations), which were associated with larger tumor size, higher des-gamma carboxyprothrombin (DCP) levels, and significantly poorer overall survival (OS). Patients with TERT promoter mutations treated with systemic chemotherapy or transcatheter arterial chemoembolization had shorter median OS compared to those without mutations. Furthermore, higher fractional abundance of TERT mutations in ctDNA correlated with worse outcomes.

Similarly, Kim et al conducted a study on 107 treatment-naïve hepatocellular carcinoma patients to evaluate the utility of circulating tumor DNA in detecting mutations and predicting prognosis.¹⁶ They identified 25 single nucleotide variants across 12 genes, with mutations in MLH1, PTEN, and STK11 being most frequently detected. Notably, the MLH1 chr3:37025749T>A SNV was associated with advanced disease stage, higher ctDNA levels, and poorer survival, particularly in patients with high ctDNA concentrations. The study demonstrated that ctDNA analysis could reliably reflect somatic mutations in HCC tissue and that the detection of specific mutations, such as the MLH1 SNV, provided prognostic value, emphasizing the potential of ctDNA as a non-invasive biomarker in HCC management. Our study found that mutations in FGFR1 (c.396_398delTGA [p.D133del]) and FGFR3 (c.1206delC [p.K403fs*29]) were associated with poor prognosis, while BRAF and KIT mutations were linked to better survival outcomes. These findings support the idea that mutations in certain genes, like FGFR1 and FGFR3, may drive aggressive tumor behavior and metastasis, whereas others, like BRAF and KIT, may reflect less aggressive tumor biology.

Shen et al conducted a study on 895 hepatocellular carcinoma (HCC) patients divided into three cohorts to investigate the role of TP53 mutations, particularly the p.R249S hotspot mutation, in tumor progression and prognosis. Cohort 1 consisted of 260 surgically treated patients, where 141 TP53 mutations (primarily missense mutations) were detected, with R249S accounting for over 60% of these. R249S was associated with enhanced cell proliferation, migration, and poor survival outcomes. Cohorts 2 and 3 involved patients with and without surgical resection, respectively, where R249S detection in circulating tumor DNA ctDNA via droplet digital PCR was significantly linked to worse overall and progression-free survival. The study highlighted R249S as a dominant mutation in HCC, emphasizing its utility as a non-invasive biomarker for predicting prognosis, regardless of surgical treatment status.¹⁷

Ono et al analyzed 46 patients with hepatocellular carcinoma and successfully detected circulating tumor DNA (ctDNA) in 7 of these patients.¹⁸ Their findings indicated that patients in the ctDNA-positive group had significantly worse extrahepatic metastasis compared to those in the ctDNA-negative group. They also observed that tumor size was significantly larger in the ctDNA-positive group. However, they did not examine which specific genes harbored mutations. In our study, while ctDNA positivity in liquid biopsy was associated with worse outcomes, this finding did not reach statistical significance. Additionally, we did not identify a correlation between ctDNA positivity and either

metastasis or tumor size. This discrepancy may be attributed to differences in genetic variants between the study populations. The detection of specific genetic mutations in ctDNA, such as those in FGFR1 and FGFR3, may be more closely associated with metastasis and tumor progression, highlighting the need for comprehensive mutation analysis in future studies.

Cai et al conducted a comprehensive study to evaluate the prognostic value of ctDNA using plasma samples from 34 long-term follow-up HCC patients.¹⁹ Their findings revealed that ctDNA could detect tumor recurrence 4.6 months earlier than imaging modalities and had greater sensitivity than conventional serum biomarkers like AFP, AFP-L3%, and DCP. Combining ctDNA with DCP enhanced minimal residual disease detection and prognostic prediction, suggesting ctDNA as a promising tool for non-invasive monitoring, improving postoperative management, and guiding therapeutic decisions in HCC.

Lehrich et al emphasize the growing significance of molecular diagnostic techniques in the management of hepatocellular carcinoma (HCC). Liquid biopsies, which analyze components such as circulating tumor cells (CTCs), extracellular vesicles (EVs), and cell-free DNA (cfDNA), offer a minimally invasive alternative for examining the genetic characteristics of tumors. However, challenges like sensitivity, cost, and accessibility remain critical barriers to widespread clinical adoption.²⁰

The identification of FGFR1/3 mutations in our cohort has important therapeutic implications. Currently, three FGFR inhibitors have received FDA approval for various cancers: erdafitinib for urothelial carcinoma with FGFR3 alterations (achieving 35.3% objective response rate),²¹ pemigatinib for cholangiocarcinoma with FGFR2 fusions (36% response rate)²² and myeloid/lymphoid neoplasms with FGFR1 rearrangements, and futibatinib for intrahepatic cholangiocarcinoma with FGFR2 rearrangements²³ (42% response rate). Although these agents are not yet approved specifically for HCC, clinical trials are investigating FGFR inhibitors in hepatobiliary malignancies. Notably, the ongoing Phase II trial (NCT04828486) is evaluating futibatinib combined with pembrolizumab in FGF19-positive advanced HCC, building on preclinical evidence of synergy between FGFR inhibition and immunotherapy.²⁴ The detection of FGFR mutations through liquid biopsy could potentially identify HCC patients who may benefit from targeted therapies, either within clinical trials or through off-label use based on molecular tumor board recommendations.

Despite the promising findings in our study, several limitations must be considered. The small sample size (n=20) and single-center design limit the generalizability of our results. Additionally, the use of serum-derived cfDNA, which may contain lower concentrations of tumor DNA compared to plasma, and the exclusion of non-coding regions (such as the TERT promoter) from our targeted panel may have reduced the sensitivity of mutation detection. This may have resulted in missed mutations relevant to HCC biology. Unlike previous studies, we did not perform matched tissue biopsies to validate ctDNA findings, which limits our ability to distinguish true tumor mutations from clonal hematopoiesis.

Future multi-center studies with larger cohorts, longitudinal sampling, and whole-exome sequencing are needed to validate the survival associations of specific mutations and to further explore the functional relevance of KIT and non-V600 BRAF variants. Furthermore, integrating ctDNA analysis with radiomics and immunotherapy response metrics may enhance the precision of personalized surveillance and therapeutic strategies in HCC.

Conclusion

In conclusion, this study highlights the potential of liquid biopsy as a valuable, non-invasive tool for the genetic profiling and monitoring of hepatocellular carcinoma (HCC). By analyzing cell-free DNA (cfDNA) from patient serum, we were able to detect key mutations associated with tumor progression and prognosis, such as those in the FGFR1, FGFR3, KIT, and BRAF genes. While these findings were based on a relatively small cohort, they suggest that specific mutations, particularly in the FGFR family and KIT, may serve as important biomarkers for predicting survival outcomes and guiding personalized therapeutic strategies.

The presence of FGFR1 and FGFR3 mutations in our study was notably linked to poorer survival, emphasizing the crucial role of the fibroblast growth factor (FGF) signaling pathway in promoting cancer progression and metastasis. Given the involvement of FGFRs in tumor cell growth, angiogenesis, and resistance to treatment, the detection of these mutations could offer a potential target for pan-FGFR inhibition in HCC, which is currently under investigation in clinical trials. Similarly, mutations in KIT and BRAF genes, while associated with better survival in our study, point to the need for further research to fully understand their role in HCC. Understanding how these mutations impact tumor

behavior and metastasis is critical for identifying potential therapeutic options, including targeted therapies that could improve patient outcomes.

Ultimately, integrating liquid biopsy with other molecular diagnostic tools, such as radiomics and immunotherapy response biomarkers, holds great promise for refining personalized treatment approaches. By combining genetic data with clinical and imaging features, we may be able to develop more tailored and effective surveillance strategies that address the unique genetic landscape of each patient's tumor, thereby improving prognosis and survival outcomes.

In summary, while liquid biopsy remains an evolving technology, its potential to provide non-invasive, real-time insights into the genetic profile of HCC makes it an invaluable asset in the ongoing fight against this challenging and aggressive cancer. Future research will undoubtedly continue to elucidate the role of specific genetic mutations in HCC progression, paving the way for more precise and effective treatment strategies.

Data Sharing Policy

The data generated and analyzed during the current study are available from the corresponding author on reasonable request.

Ethical Approval

Ethical approval was obtained from the Çukurova University Faculty of Medicine Ethics Committee for Non-Invasive Clinical Research (Decision No. 12; February 14, 2020).

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Disclosure

There are no conflicts of interest to declare.

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