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# Circulating tumor DNA (ctDNA) as a biomarker of response to therapy in advanced Hepatocellular carcinoma treated with Nivolumab

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**Abstract.**
**BACKGROUND:** Circulating tumor DNA (ctDNA) is a promising non-invasive marker for detection, diagnosis, treatment selection, and prognosis of hepatocellular carcinoma (HCC).
**OBJECTIVE:** This study aimed to examine the utility of ctDNA as a prognostic and predictive tool in HCC patients treated with nivolumab.

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**METHODS:** We analyzed pre-treatment ctDNA from 44 HCC patients using comprehensive genomic testing on a commercially available platform. We utilized log rank test and univariate Cox models to correlate overall survival (OS) and progression-free survival (PFS) with ctDNA expressions.
**RESULTS:** Of 44 patients, 77.3% were men with median age of 67 years. All but 3 patients had at least one alteration identified, and *TP53* was the most commonly altered gene (52.3%). Median OS was 17.5 months (95% CI: 12.7, NA). Mutations involving *PIK3CA*, *BRCA1*, and *CCND1* amplification were associated with shorter OS (P 0.0001, 0.0001 and 0.01, respectively). Median PFS time was 4.01 months (95% CI: 3.06, 9.33). Mutations involving *KIT* and *PIK3CA* were associated with shorter PFS (P 0.0001 and 0.0004, respectively), while mutation involving *CTNNB1* were associated with longer PFS ($p = 0.04$).
**CONCLUSIONS:** ctDNA profiling may provide a benefit for prediction of survival and progression of HCC patients treated with nivolumab. Future studies are needed for confirmation.

Keywords: ctDNA, hepatocellular carcinoma, immunotherapy, nivolumab, biomarker

## 1. Introduction

Hepatocellular carcinoma (HCC) is one of the most lethal malignancies and a leading cause of cancer related mortality worldwide [1,2]. Despite the recent advances in treatment of advanced HCC; The prognosis remains poor compared to patients diagnosed and treated at early stage. In addition, higher frequency of recurrence could occur after both local and systemic treatment [3].

Such poor prognosis for HCC patients represents a serious clinical problem, which could be attributed to the absence of specific symptoms in early stages, lack of accurate markers and tools for early diagnosis, treatment selection and outcome prediction; which leads to most patients often diagnosed at an advanced stage and suffering poor outcome. Thus, early diagnosis of HCC and accurate treatment strategies are highly important to improve HCC prognosis [4,5].

Circulating tumor DNA (ctDNA) is the fraction of cell-free DNA(cfDNA) derived from primary or metastatic tumors; it has emerged as a potential non-invasive markers and is starting to be adopted in clinical practice to detect mutations and to monitor disease course for several major cancer types [6].

In HCC, ctDNA has proven beneficial for tracking traces of tumors in high risk population, detecting early stage and detection of genomic changes in advanced HCC patients [7,8]. Studies have shown that ctDNA levels correlate with tumor burden and disease progression, making it a valuable tool for prognosis. ctDNA has been found to be inversely correlated with poor prognosis and shorter overall survival (OS) [9]. Given the increasing application of immune checkpoint inhibitors such as nivolumab in advanced HCC [10], understanding ctDNA alterations can provide valuable insights into treatment outcomes and guide therapeutic decisions. Our study aims to investigate whether pre-treatment ctDNA alterations can serve as prognostic biomarkers in HCC patients treated with nivolumab, thus potentially aiding in patient stratification and personalized treatment planning.

Recent studies have demonstrated that changes in ctDNA levels are associated with treatment outcomes in various cancers, including HCC. Specifically, a reduction in ctDNA levels has been correlated with improved progression-free survival (PFS) and overall survival (OS) in patients receiving immune checkpoint inhibitors. This highlights the potential of ctDNA as a non-invasive biomarker for monitoring and predicting patient responses to immunotherapy [11,12].

We specifically selected a population of HCC patients treated with nivolumab due to its growing use and promising results in managing advanced HCC. Nivolumab, an anti-PD-1 antibody, has shown significant efficacy and manageable safety in advanced HCC, making it a critical component of current treatment strategies [13]. By focusing on this patient population, we aim to provide insights into how ctDNA alterations can inform and potentially predict responses to immunotherapy, addressing a significant clinical need for predictive biomarkers in this context.

In the current study, we assessed the prognostic significance of baseline ctDNA in patients with HCC treated with nivolumab (anti-PD-1). This investigation represents the first prospective study of pre-treatment ctDNA association with OS and progression free survival (PFS) among patients with HCC treated with nivolumab.

## 2. Patients and methods

The study was approved by the University of Texas MD Anderson Cancer Center's Institutional Review Board, and informed consent was obtained from all pa-

tients. We prospectively collected Blood samples from 44 HCC patients who were treated with nivolumab and followed up until progression and/or death and analyzed correlation with pretreatment ctDNA expressions. Adult patients with pathologically or radiologically confirmed HCC, as defined by the American Association for the Study of Liver Diseases, who were treated at MD Anderson Cancer Center (MDACC) from December 2017 to May 2020 and had ctDNA results available were included in the study. We accessed medical records for research purposes of these patients between December 2017 and July 2021 for information regarding medical conditions, clinical parameters, including pretreatment of ctDNA expressions, and survival outcomes.

Patients' blood samples and epidemiologic and clinical data were collected, and blood samples were analyzed retrospectively for ctDNA expressions [14,15]. Clinical and epidemiological data were retrieved from medical records. PFS was calculated from the date that Nivolumab treatment began to the date of disease progression or death, whichever occurred first. OS was calculated from the date that Nivolumab treatment began to the date of death or to the date of the last follow-up visit. The Kaplan-Meier method was used to calculate the time to event outcomes (i.e., OS and PFS) with Log rank test to compare OS or PFS between subgroups [16]. This study was approved by MD Anderson Cancer Center's Institutional Review Board.

### 2.1. ctDNA analysis

Blood-samples were shipped to a Clinical Laboratory Improvement Act (CLIA)-certified, College of American Pathologists-accredited laboratory (Guardant Health, Redwood City, California) and where ctDNA was analyzed. The ctDNA was extracted and analyzed using a comprehensive genomic testing platform. This platform provided detailed genomic profiling, focusing on genes frequently mutated in HCC, including TP53, PIK3CA, BRCA1, CCND1, and CTNNB1. The selected panel was chosen due to its relevance in cancer biology and its potential to provide insights for patient.

### 2.2. Statistical analysis

Continuous patient characteristics were summarized using descriptive statistics, categorical patient characteristics were tabulate with frequency and percentage. Fisher's exact test and Wilcoxon rank sum test were used to evaluate the association between response and ctDNA expressions [17,18]. Log rank test and univariate Cox models were used to evaluate the association between OS or PFS and ctDNA [19].

Table 1
Patient demographics and characteristics

| Characteristics | N (%) |
|---|---|
| Age, y, median | 67 |
| Range | 36–81 |
| Sex | |
| Female | 10 (22.7%) |
| Male | 34 (77.3%) |
| Race | |
| White or Caucasian | 27 (61.4%) |
| Black or African American | 4 (9.1%) |
| Asian | 7 (15.9%) |
| Other | 5 (11.4%) |
| Unknown | 1 (2.3%) |
| Ethnicity | |
| Hispanic or Latino | 6 (13.6%) |
| Non Hispanic or Latino | 36 (81.8%) |
| Unknown | 2 (4.5%) |
| Number of prior systemic therapies | |
| 1 | 44 (93.2%) |
| 2 | 3 (6.8%) |
| Number of mutations | |
| 0 | 3 (6.8%) |
| 1 | 13 (29.5%) |
| 2 | 14 (31.8%) |
| 3 | 8 (18.2%) |
| 5 | 3 (6.8%) |
| 6 | 2 (4.5%) |
| 8 | 1 (2.3%) |
| Cirrhosis | |
| No | 3 (6.8%) |
| Present | 41 (93.2%) |
| Etiology | |
| HBV | 5 (11.4%) |
| HCV | 14 (31.8%) |
| HCV/HBV | 3 (6.8%) |
| NASH | 13 (29.5%) |
| NASH/hemochromatosis | 1 (2.3%) |
| NASH/ALD | 1 (2.3%) |
| ALD | 2 (4.6%) |
| Hemochromatosis | 1 (2.3%) |
| N/A | 4 (9.1%) |
| Child_Pugh_group | |
| A | 33 (73.3%) |
| B | 11 (24.4%) |
| Child_Pugh_Score | |
| 5 | 25 (10.2%) |
| 6 | 8 (16.3%) |
| 7 | 6 (12.2%) |
| 8 | 4 (8.2%) |
| 9 | 1 (2%) |
| Pathology | |
| Hepatocellular carcinoma | 41 (93.2%) |
| Not available | 3 (6.8%) |
| Differentiation | |
| Not available | 8 (18.2%) |
| Well differentiated | 6 (13.6%) |
| Well to mod differentiated | 1 (2.3%) |
| Mod differentiated | 21 (47.7%) |
| Mod to poorly differentiated | 2 (4.5%) |
| -+Poorly differentiated | 6 (13.6%) |
| Nodularity | |
| Unimodular | 3 (6.8%) |
| Multinodular | 41 (93.2%) |

Table 1, continued

| Characteristics | N (%) |
|---|---|
| Vascular_invasion | |
| None | 18 (40.9%) |
| Present | 26 (59.1%) |
| Metastasis | |
| None | 19 (43.2%) |
| Present | 25 (56.8%) |
| HCV | |
| Negative | 26 (59.1%) |
| positive | 17 (38.6%) |
| Not available | 1 (2.3%) |
| HBsAg | |
| Negative | 36 (81.8%) |
| positive | 8 (18.2%) |
| AntiHBc | |
| Negative | 35 (79.5%) |
| positive | 9 (20.5%) |
| HIV | |
| Negative | 44 (100%) |
| Diabetes | |
| Negative | 24 (54.5%) |
| Positive | 20 (45.5%) |
| Alcohol | |
| Negative | 30 (68.2%) |
| Positive | 14 (31.8%) |
| Image response | |
| No | 31 (70.5%) |
| Yes | 9 (20.5%) |
| Not available | 4 (9.1%) |
| Disease_control | |
| No | 28 (63.6%) |
| Yes | 14 (31.8%) |
| Not available | 2 (4.5%) |

Abbreviations: Y: Year, N/A: not applicable, ALD: Alcoholic liver disease, NASH: nonalcoholic steatohepatitis.

## 3. Results

The study included 44 patients with advanced HCC who received nivolumab at MD Anderson Cancer Center and had ctDNA available prior to the start of treatment. The demographic characteristics of these patients (Table 1) were documented at the time of nivolumab administration; Median age at the time of diagnosis was 67 years (range: 36–81), 34 patients were male patients, 10 were female patients, 27 patients were white, 4 were African American, and 7 were Asian. Thirty-three (73.3%) patients were in Child-Pugh A stage and 11 (24.2%) were in Child-Pugh B. Fourteen patients tested positive for HCV, 5 tested HBV positive, and 3 tested positive for both HCV and HBV. ctDNA analysis identified at least 1 alteration in 41/44 (93.2%) of the patients. The median number of alterations/patient was 2 (range, 0–8). *TP53* was the most common altered gene ($n = 23$) followed by *CTNBB1* ($n = 19$),

Table 2
ctDNA detection and mutation number

| Mutation | Mutation detected (Yes/No) | Frequency (%) |
|---|---|---|
| CTNNB1 | No | 28 (63.6%) |
| | Yes | 16 (36.4%) |
| TP53 | No | 21 (47.7%) |
| | Yes | 23 (52.3%) |
| TERT | No | 32 (72.7%) |
| | Yes | 12 (27.3%) |
| KRAS | No | 41 (93.2%) |
| | Yes | 3 (6.8%) |
| GNAS | No | 43 (97.7%) |
| | Yes | 1 (2.3%) |
| CCND1_AMPL | No | 41 (93.2%) |
| | Yes | 3 (6.8%) |
| CCNE1_AMPL | No | 43 (97.7%) |
| | Yes | 1 (2.3%) |
| NFE2L2 | No | 42 (95.5%) |
| | Yes | 2 (4.5%) |
| KIT | No | 43 (97.7%) |
| | Yes | 1 (2.3%) |
| PIK3CA | No | 43 (97.7%) |
| | Yes | 1 (2.3%) |
| EGFR | No | 42 (95.5%) |
| | Yes | 2 (4.5%) |
| RAF1 | No | 43 (97.7%) |
| | Yes | 1 (2.3%) |
| RB1 | No | 38 (86.4%) |
| | Yes | 6 (13.6%) |
| ALK | No | 43 (97.7%) |
| | Yes | 1 (2.3%) |
| NF1 | No | 41 (93.2%) |
| | Yes | 3 (6.8%) |
| BRAF | No | 43 (97.7%) |
| | Yes | 1 (2.3%) |
| NTRK1 | No | 43 (97.7%) |
| | Yes | 1 (2.3%) |
| APC | No | 43 (97.7%) |
| | Yes | 1 (2.3%) |
| FGFR1 | No | 43 (97.7%) |
| | Yes | 1 (2.3%) |
| FGFR2 | No | 43 (97.7%) |
| | Yes | 1 (2.3%) |
| FGFR3 | No | 43 (97.7%) |
| | Yes | 1 (2.3%) |
| ARIDA1A | No | 41 (93.2%) |
| | Yes | 3 (6.8%) |
| BRCA2 | No | 42 (95.5%) |
| | Yes | 2 (4.5%) |
| MET | No | 38 (86.4%) |
| | Yes | 6 (13.6%) |
| HIF1A | No | 43 (97.7%) |
| | Yes | 1 (2.3%) |
| PDGFRA | No | 42 (95.5%) |
| | Yes | 2 (4.5%) |
| CDKN2A | No | 43 (97.7%) |
| | Yes | 1 (2.3%) |
| FBXW7 | No | 43 (97.7%) |
| | Yes | 1 (2.3%) |
| NOTCH1 | No | 43 (97.7%) |
| | Yes | 1 (2.3%) |
| BRCA1 | No | 43 (97.7%) |
| | Yes | 1 (2.3%) |
| MTOR | No | 42 (95.5%) |
| | Yes | 2 (4.5%) |
| ESR | No | 43 (97.7%) |
| | Yes | 1 (2.3%) |

Table 3
Log rank test to evaluate the association between ctDNA categorical data and OS

| Variable name | Level | N | Event | Median OS (95%CI) (M) | OS Rate at 1 Year (95%CI) | $P$-value |
|---|---|---|---|---|---|---|
| | All patients | 44 | 17 | 17.51 (12.71, NA) | 0.67 (0.53, 0.84) | |
| CTNNB1 | No | 28 | 13 | 17.51 (11.53, NA) | 0.62 (0.45, 0.85) | 0.37 |
| | Yes | 16 | 4 | NA (12.71, NA) | 0.75 (0.54, 1) | |
| TP53 | No | 21 | 8 | 18.81 (12.71, NA) | 0.7 (0.51, 0.97) | 0.89 |
| | Yes | 23 | 9 | 13.93 (11.53, NA) | 0.64 (0.46, 0.9) | |
| TERT | No | 32 | 13 | 20.11 (11.53, NA) | 0.65 (0.49, 0.87) | 0.66 |
| | Yes | 12 | 4 | 17.51 (17.51, NA) | 0.73 (0.51, 1) | |
| KRAS | No | 41 | 15 | 20.11 (12.71, NA) | 0.67 (0.52, 0.85) | 0.63 |
| | Yes | 3 | 2 | 17.51 (7.06, NA) | 0.67 (0.3, 1) | |
| GNAS | No | 43 | 16 | 20.11 (13.83, NA) | 0.69 (0.56, 0.87) | 0.32 |
| | Yes | 1 | 1 | 11.53 (NA, NA) | NA | |
| NFE2L2 | No | 42 | 16 | 17.51 (12.71, NA) | 0.68 (0.53, 0.86) | 0.59 |
| | Yes | 2 | 1 | 2.99 (2.99, NA) | 0.5 (0.13, 1) | |
| PIK3CA | No | 43 | 16 | 17.51 (13.83, NA) | 0.69 (0.54, 0.86) | $< 0.0001$ |
| | Yes | 1 | 1 | 3.88 (NA, NA) | NA | |
| RAF1 | No | 43 | 16 | 20.11 (13.83, NA) | 0.69 (0.55, 0.86) | 0.2 |
| | Yes | 1 | 1 | 9.89 (NA, NA) | NA | |
| RB1 | No | 38 | 14 | 20.11 (12.71, NA) | 0.69 (0.54, 0.87) | 0.31 |
| | Yes | 6 | 3 | 13.93 (10.78, NA) | 0.53 (0.21, 1) | |
| MET | No | 38 | 15 | 17.51 (12.71, NA) | 0.68 (0.54, 0.87) | 0.4 |
| | Yes | 6 | 2 | NA (7.06, NA) | 0.56 (0.23, 1) | |
| BRCA1 | No | 43 | 16 | 17.51 (13.83, NA) | 0.68 (0.54, 0.86) | $< 0.0001$ |
| | Yes | 1 | 1 | 2.99 (NA, NA) | NA | |
| MTOR | No | 42 | 16 | 17.51 (13.83, NA) | 0.68 (0.54, 0.86) | 0.45 |
| | Yes | 2 | 1 | 7.85 (NA, NA) | NA | |
| CCND1_AMPL | No | 41 | 15 | 20.11 (13.83, NA) | 0.71 (0.57, 0.88) | 0.01 |
| | Yes | 3 | 2 | 11.53 (2.99, NA) | NA | |

Abbreviations: N: number; OS: overall survival; N/A: not applicable.

*TERT* ($n = 12$), *MET* ($n = 6$) and *RB1* ($n = 6$). Other mutations were infrequently present: *KRAS*, *EGFR*, *ARIDA1A*, *NF1* and *CCND1* amplification in three tumors; BRCA2, NFE2L2, EGFR and *PDGFRA* in two tumors, and *GNAS*, *CCNE1*, *KIT*, *PIK3CA*, *ALK*, *BRAF*, *NTRK1*, *APC* and *FGFR1* in only one tumor among others. Table 2.

The median OS was 17.5 months (95% CI: 12.7, NA The estimated median follow-up time was 14.7 months (95% CI: 12.7, 19.0). Mutations involving *PIK3CA*, *BRCA1*, and *CCND1* amplification were associated with shorter OS (P 0.0001, 0.0001, 0.01, respectively. Table 3).

The median PFS time was 4.01 months (95% CI: 3.06, 9.33). Forty-three patients were available for PFS analysis, and 37 of the 43 patients had PFS events (death or PD whichever occurred first). Mutations involving *KIT* and *PIK3CA* were associated with shorter PFS (P 0.0001 and 0.0004, respectively), while mutations involving *CTNNB1* were associated with longer PFS ($p = 0.04$). No significant differences in OS or PFS was observed for other alterations (Table 4).

## 4. Discussion

In this study, we investigated baseline ctDNA as a prognostic biomarker in advanced HCC patients treated with nivolumab. We assessed pretreatment ctDNA plasma samples from 44 patients. Whereas the small cohort size is considered a limitation of this study, we have demonstrated the predictive value and the utility of ctDNA as a clinical biomarker.

Nivolumab, an anti–programmed cell death 1 (anti-PD-1) checkpoint inhibitors nivolumab, has been approved for the treatment of HCC patients. In CheckMate 040, nivolumab monotherapy demonstrated manageable safety, objective response rate (ORR) of 14%, duration of response of at least 12 months in 59% of patients, and promising long-term median survival of 15.1 months in patients with advanced HCC treated with sorafenib [20].

In addition to comorbidities and tumor burden, selecting the most effective treatment for advanced HCC has to be carefully for the risk-benefit ratio. Systemic therapies for advanced HCC usually incur a high-cost burden on both patients and healthcare system. It is

Table 4
Log rank test to evaluate the association between ctDNA categorical data and PFS

| Varname | Level | N | Event | Median PFS (95%CI) (M) | PFS Rate at 6 m (95%CI) | $P$-value |
|---|---|---|---|---|---|---|
| | All patients | 43 | 37 | 4.01 (3.06, 9.33) | 0.39 (0.27, 0.57) | |
| CTNNB1 | No | 27 | 25 | 3.45 (2.92, 8.61) | 0.28 (0.15, 0.52) | 0.04 |
| | Yes | 16 | 12 | 7.42 (3.38, NA) | 0.56 (0.37, 0.87) | |
| TP53 | No | 21 | 19 | 3.48 (2.92, 10.84) | 0.29 (0.15, 0.56) | 0.32 |
| | Yes | 22 | 18 | 5.09 (3.06, 14.13) | 0.49 (0.32, 0.76) | |
| TERT | No | 31 | 27 | 3.68 (3.06, 10.84) | 0.38 (0.24, 0.6) | 0.78 |
| | Yes | 12 | 10 | 4.62 (2.3, NA) | 0.42 (0.21, 0.81) | |
| KRAS | No | 40 | 35 | 3.68 (3.06, 9.33) | 0.39 (0.27, 0.58) | 0.95 |
| | Yes | 3 | 2 | 4.14 (2.3, NA) | 0.33 (0.07, 1) | |
| GNAS | No | 42 | 36 | 4.01 (3.06, 9.33) | 0.4 (0.27, 0.58) | 0.55 |
| | Yes | 1 | 1 | 3.45 (NA, NA) | NA | |
| NFE2L2 | No | 41 | 36 | 4.01 (3.22, 9.33) | 0.38 (0.26, 0.57) | 0.76 |
| | Yes | 2 | 1 | 2.99 (2.99, NA) | 0.5 (0.13, 1) | |
| KIT | No | 42 | 36 | 4.01 (3.22, 9.33) | 0.4 (0.27, 0.58) | $< 0.0001$ |
| | Yes | 1 | 1 | 1.84 (NA, NA) | NA | |
| PIK3CA | No | 42 | 36 | 4.01 (3.22, 9.33) | 0.4 (0.27, 0.58) | $P = 0.0004$ |
| | Yes | 1 | 1 | 1.91 (NA, NA) | NA | |
| EGFR | No | 41 | 35 | 3.68 (3.06, 9.33) | 0.36 (0.24, 0.54) | 0.83 |
| | Yes | 2 | 2 | 9.07 (8.61, NA) | 1 (1, 1) | |
| RAF1 | No | 42 | 36 | 4.01 (3.06, 9.33) | 0.4 (0.27, 0.58) | 0.61 |
| | Yes | 1 | 1 | 3.48 (NA, NA) | NA | |
| RB1 | No | 38 | 32 | 3.68 (2.99, 9.53) | 0.41 (0.28, 0.61) | 0.63 |
| | Yes | 5 | 5 | 4.01 (3.22, NA) | 0.2 (0.03, 1) | |
| ALK | No | 42 | 36 | 4.01 (3.22, 9.33) | 0.4 (0.27, 0.58) | 0.4 |
| | Yes | 1 | 1 | 3.06 (NA, NA) | NA | |
| NF1 | No | 40 | 34 | 4.01 (3.06, 9.33) | 0.39 (0.27, 0.58) | 0.6 |
| | Yes | 3 | 3 | 3.38 (2.73, NA) | 0.33 (0.07, 1) | |
| BRAF | No | 42 | 36 | 4.01 (3.06, 9.33) | 0.4 (0.27, 0.58) | 0.5 |
| | Yes | 1 | 1 | 3.38 (NA, NA) | NA | |
| APC | No | 42 | 36 | 3.68 (3.06, 9.53) | 0.37 (0.25, 0.55) | 0.88 |
| | Yes | 1 | 1 | 9.33 (NA, NA) | 1 (1, 1) | |
| FGFR2 | No | 42 | 36 | 3.68 (3.06, 9.53) | 0.37 (0.25, 0.55) | 0.88 |
| | Yes | 1 | 1 | 9.33 (NA, NA) | 1 (1, 1) | |
| FGFR3 | No | 42 | 36 | 3.68 (3.06, 9.53) | 0.37 (0.25, 0.55) | 0.88 |
| | Yes | 1 | 1 | 9.33 (NA, NA) | 1 (1, 1) | |
| ARIDA1A | No | 40 | 35 | 3.68 (3.06, 8.61) | 0.37 (0.24, 0.55) | 0.77 |
| | Yes | 3 | 2 | 9.33 (2.76, NA) | 0.67 (0.3, 1) | |
| BRCA2 | No | 41 | 35 | 4.01 (3.22, 9.53) | 0.38 (0.26, 0.57) | 0.44 |
| | Yes | 2 | 2 | 5.58 (1.84, NA) | 0.5 (0.13, 1) | |
| MET | No | 37 | 31 | 4.14 (3.45, 10.84) | 0.42 (0.29, 0.62) | 0.06 |
| | Yes | 6 | 6 | 3.02 (2.3, NA) | 0.17 (0.03, 1) | |
| HIF1A | No | 42 | 36 | 3.68 (3.06, 9.53) | 0.37 (0.25, 0.55) | 0.98 |
| | Yes | 1 | 1 | 7.42 (NA, NA) | 1 (1, 1) | |
| PDGFRA | No | 41 | 35 | 3.68 (3.06, 9.33) | 0.36 (0.24, 0.54) | 0.12 |
| | Yes | 2 | 2 | 64.77 (8.61, NA) | 1 (1, 1) | |
| CDKN2A | No | 42 | 36 | 4.01 (3.22, 9.33) | 0.4 (0.27, 0.58) | 0.35 |
| | Yes | 1 | 1 | 2.99 (NA, NA) | NA | |
| FBXW7 | No | 42 | 36 | 4.01 (3.22, 9.33) | 0.4 (0.27, 0.58) | 0.35 |
| | Yes | 1 | 1 | 2.99 (NA, NA) | NA | |
| NOTCH1 | No | 42 | 36 | 4.01 (3.22, 9.33) | 0.4 (0.27, 0.58) | 0.35 |
| | Yes | 1 | 1 | 2.99 (NA, NA) | NA | |
| BRCA1 | No | 42 | 36 | 4.01 (3.22, 9.33) | 0.4 (0.27, 0.58) | 0.35 |
| | Yes | 1 | 1 | 2.99 (NA, NA) | NA | |
| MTOR | No | 41 | 36 | 4.01 (3.22, 9.33) | 0.38 (0.26, 0.57) | 0.97 |
| | Yes | 2 | 1 | 1.84 (1.84, NA) | 0.5 (0.13, 1) | |
| CCND1_AMPL | No | 40 | 34 | 4.14 (3.22, 9.53) | 0.42 (0.29, 0.6) | 0.17 |
| | Yes | 3 | 3 | 3.06 (2.99, NA) | NA | |
| CCNE1_AMPL | No | 42 | 36 | 3.68 (3.06, 9.53) | 0.37 (0.25, 0.55) | 0.88 |
| | Yes | 1 | 1 | 9.33 (NA, NA) | 1 (1, 1) | |

Abbreviations: N: number; PFS: Progression-free survival; N/A: not applicable.

important to know which patients will benefit from nivolumab versus those in whom it will have lower survival benefit. Despite the recent breakthrough in the treatment of patients with advanced HCC, there remains an unmet need for reliable biomarkers of response to immunotherapy. ctDNA have been reported to be a prognostic biomarker in HCC patients [9,12,21].

Our group recently reported the mutational landscape of HCC tumorigenesis for the purpose of selecting patients for targeted therapy trials and the potential clinical utility of ctDNA [22], The total number of alterations was 680 (nonunique); median number of alterations/patient was three (range, 1–13); median mutant allele frequency (% cfDNA), 0.49% (range, 0.06%–55.03%). *TP53* was the most commonly altered gene [$>$ 120 alterations (non-unique)] followed by *EGFR, MET, ARID1A, MYC, NF1, BRAF, and ERBB2* [20–38 alterations (nonunique)/gene]. Of the patients with alterations, 56.9% (103/181) had $\geqslant$ 1 actionable alterations, most commonly in *MYC, EGFR, ERBB2, BRAF, CCNE1, MET, PIK3CA, ARID1A, CDK6, and KRAS*. In these genes, amplifications occurred more frequently than mutations. Hepatitis B (HBV)-positive patients were more likely to have *ERBB2* alterations, 35.7% (5/14) versus 8.8% in HBV-negative patients ($P$ = 0.04) [22].

In this study, we demonstrated the potential clinical utility of ctDNA in patients treated with Nivolumab and the prognostic value of ctDNA for patients in real-world clinical practice. Our study is the first to report ctDNA association with outcomes among patients with HCC treated with nivolumab. Mutations involving *PIK3CA*, *BRCA1*, and *CCND1* were associated with shorter OS. Mutations involving *KIT* and *PIK3CA* were associated with shorter PFS, while mutation involving *CTNNB1* were associated with longer PFS.

The clinical significance of our study lies in its potential to improve personalized treatment strategies for advanced HCC. By identifying ctDNA alterations that correlate with survival outcomes, clinicians can better stratify patients based on their likelihood of responding to nivolumab. This can lead to more tailored treatment plans, optimizing the use of immunotherapy and potentially improving patient outcomes. Additionally, the use of ctDNA as a non-invasive biomarker offers a practical tool for ongoing monitoring of treatment response and disease progression, reducing the need for more invasive procedures.

Our study had some limitation. Firstly, this investigation was conducted at a single center study. Moreover, the small sample size of patients included in the study. Whereas *PIK3CA* and *BRCA1* were associated with a shorter OS, *KIT* and *PIK3CA* were also associated with shorter PFS; Hence, these observations need to be further explored and validated in a lager cohort of HCC patients.

Secondly, given the low incidence of molecular alterations in HCC the current study which was exploratory and retrospective, the study was not powered. Lastly, we carried out ctDNA sequencing using commercially available kit (Guardian Health, CA), which only allowed us to study a limited number of genes. Thus, the study is not very inclusive of all genes and alterations. Therefore, herein we focused on any alteration and its relations with the outcome irrespective of the gene being actionable or non-actionable. A larger sample size with enough power is required to validate these observations and further confirm our results.

To the best of our knowledge, this is the first study to assess clinical prognostic value of ctDNA in patients who have received Nivolumab in clinical setting. Our results provide evidence to suggest the clinical utility of ctDNA as a noninvasive biomarker for prediction of OS and PFS in patients with HCC treated with nivolumab and potentially other immunotherapy approaches.

Although this study focused on its baseline prognostic value, serial ctDNA evaluation in future studies is expected have prognostic significance and provide useful data to eventually guide therapy decision in clinical routine practice. This study provides a strong argument for the use of ctDNA as a personalized clinical tool. It has the potential to serve as a prognostic tool for advanced HCC patients and may guide treatment choices. Future studies in larger randomized clinical trial are warranted to further validate these findings.

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## Conflict of interest

All authors report no commercial associations (e.g., consultancies, stock ownership, equity interests, or patent-licensing arrangements) that might pose a conflict of interest in connection with the submitted article.

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