Feasibility of Personalized and Tumor-Informed Circulating Tumor DNA Assay for Early Recurrence Detection in Patients With Hepatocellular Carcinoma

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ABSTRACT

PURPOSE Hepatocellular carcinoma (HCC) has high relapse rates after standard-of-care (SOC) resection or liver transplantation (LT). We evaluated the utility of circulating tumor DNA (ctDNA) to predict relapse/progression risk in patients with HCC.

MATERIALS AND METHODS This retrospective analysis examined real-world data from ctDNA testing on 125 patients with HCC (721 plasma samples) undergoing curative-intent treatments and SOC management. Patients were divided into four subcohorts: cohort A (n = 64) and B (n = 52) comprised patients under recurrence monitoring after LT or resection, respectively. Cohort C (n = 4) and D (n = 5) comprised patients under treatment response monitoring with known recurrence or inoperable disease, respectively. A personalized, tumor-informed 16-plex polymerase chain reaction next-generation sequencing assay (Signatera, Natera, Inc, Austin, TX) was used for ctDNA testing. The molecular residual disease (MRD) window was defined as 2-12 weeks post-LT/resection (cohorts A/B), before starting adjuvant therapy (AT). Surveillance window was defined as post-MRD window or 2 weeks post-AT (cohort B) or during ongoing treatment (cohorts C/D).

RESULTS The median follow-up was 40 (1.5-60) months. In cohort A, 97.2% (35/36) of patients with ctDNA negativity in the MRD window remained negative during surveillance. In cohort B, ctDNA was detected in 29.4% (10/34) of patients within the MRD window, all of whom experienced clinical recurrence (hazard ratio [HR], 7.2 [95% CI, 2.6 to 20]; $P < .0001$). In the surveillance window (cohort B), the ctDNA detection rate was 32.3% (10/31), and all experienced recurrence (HR, 18.0 [95% CI, 3.9 to 85]; $P < .0001$). In cohorts C/D, on-treatment ctDNA dynamics were concordant with treatment response as measured by imaging. Compared with alpha-fetoprotein, ctDNA had higher sensitivity and a significantly longer lead time (7.9 *v* 2.2 months) for recurrence detection.

CONCLUSION Serial ctDNA testing effectively identified HCC recurrence early, postresection and post-LT. ctDNA was also useful for treatment response monitoring and could help resolve ambiguous imaging results.

ACCOMPANYING CONTENT

Data Sharing Statement

Data Supplement

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INTRODUCTION

Hepatocellular carcinoma (HCC) accounts for approximately 90% of primary liver cancers.[1] Despite being the seventh most common cancer globally, HCC is the second leading cause of cancer-related deaths,[2] with a 5-year overall survival rate of 18%.[3] Surgical resection and liver transplantation (LT), when possible, remain the primary curative-intent treatment options.[4] For patients with unresectable tumors because of location, tumor burden, or inadequate hepatic reserve, LT is the preferred option.[5-16] However, up to 20% of patients experience recurrence within 5 years of transplantation,[17,18] and recurrence is higher (>50%) in patients who undergo hepatectomy only.[19,20] Post-LT recurrence is often aggressive, difficult to manage in immunosuppressed transplant recipients, and associated

CONTEXT

Key Objective

Circulating tumor DNA (ctDNA) is a promising biomarker for monitoring molecular residual disease and predicting recurrence in hepatocellular carcinoma (HCC). This retrospective analysis of real-world data evaluated the utility of ctDNA in predicting recurrence after curative-intent treatment (liver transplantation or surgical resection).

Knowledge Generated

ctDNA positivity post-treatment was associated with increased recurrence risk. Although limited by sample size and inconsistent follow-up, these findings underscore the potential of ctDNA as a prognostic biomarker in HCC, warranting prospective validation.

Relevance

These results contribute to the growing evidence supporting ctDNA as a tool for risk stratification and postoperative surveillance in HCC.

with poor survival (median survival, 10-13 months after recurrence).[21,22]

Early recurrence detection is clinically important, as limited recurrence is treated with curative intent, following the same pathway as newly diagnosed HCC as per National Comprehensive Cancer Network (NCCN) guidelines (version 3.2024).[4] Such patients may undergo resection, radiofrequency or microwave ablations, and other liver-directed therapies,[23,24] which may improve survival.[23,25,26] Recognizing the importance of timely surgical intervention, the Working Group from the International Liver Transplantation Society (ILTS) Transplant Oncology Consensus Conference (2020) recommends close surveillance after transplantation,[25] especially during the first 2 years when recurrence risk is the highest.[21,27] Current surveillance protocols rely on imaging (ultrasound, magnetic resonance imaging [MRI], or computed tomography [CT]) in conjunction with alpha-fetoprotein (AFP) tests every 3-6 months for the first 2 years and every 6-12 months thereafter.[4] However, AFP has a relatively low sensitivity and specificity, marginally improving imaging-based surveillance.[20,28] Although CT and MRI are highly specific imaging modalities for detecting HCC, their sensitivity is limited to detect macroscopic recurrences, and earlier detection may lead to improved access to curative-intent therapeutic interventions.[29,30] More than half of the patients experience HCC recurrence only at extrahepatic sites, such as the lungs and bones, requiring CT scans of both the chest and abdomen during surveillance.[21] Therefore, reliable blood-based biomarkers are needed for detecting both local and distant recurrences.

Plasma circulating tumor DNA (ctDNA) has emerged as a minimally invasive, prognostic, and predictive biomarker across multiple cancers.[31-36] In HCC, ctDNA monitoring can predict relapse early and monitor response to adjuvant treatment after definitive surgery.[16,37-40] However, reports regarding the utility of ctDNA in monitoring HCC recurrence after liver resection or transplantation are limited. The purpose of this study was to evaluate the feasibility of a personalized, tumor-informed ctDNA assay for monitoring relapse in patients with HCC, including those who underwent curative-intent LT.

MATERIALS AND METHODS

Patient Population and Study Design

This was a retrospective analysis of real-world data from 125 patients (721 plasma time points) across 11 centers with stage I-IV HCC who underwent curative-intent treatment and clinical ctDNA testing. The diagnosis of HCC was confirmed with histopathologic examination of the explanted liver. All patients received treatment in compliance with the NCCN guidelines. The curative-intent treatments included LT, hepatectomy, and liver-directed/systemic therapy for patients with unresectable tumors.

The patients were divided into four mutually exclusive subcohorts for analysis. Cohort assignment was based on clinical scenario AND timing of first blood collection. Cohort A (n = 64, 51.2%) included patients who received curative-intent LT; cohort B (n = 52, 41.6%) included patients who underwent hepatectomy; cohort C (n = 4, 3.2%) included patients who received treatment because of HCC recurrence and the timing of ctDNA testing was after recurrence; cohort D (n = 5, 4.0%) included patients with unresectable/inoperable disease and received treatment.

Longitudinal ctDNA testing was done after the curative LT or hepatectomy performed for post-transplantation recurrence. Imaging was obtained per standard clinical practice and used to evaluate tumor response using the modified RECIST criteria.[41] Postdiagnostic AFP measurements were available for a subset of patients (n = 35, cohort B). Approval for this study was provided by the Houston Methodist

Hospital Institutional Review Board (IRB; protocol number PRO00031693), Georgetown University IRB Protocol 2016-0419, and under 45 Code of Federal Regulations 164.501 was determined to be exempt research by an independent IRB—Salus 20099—04. The study was conducted in accordance with the Declaration of Helsinki.

ctDNA Testing Using a Personalized, Tumor-Informed Assay

All biological samples for ctDNA testing were processed at Natera, Inc (Austin, TX), following a Clinical Laboratory Improvement Amendments–validated standard operating procedure. Clinically validated, personalized, tumor-informed 16-plex polymerase chain reaction (PCR) next-generation sequencing ctDNA assays (Signatera, Natera, Inc.) were ordered at the discretion of the treating physician and designed for each patient as previously described.[42] Briefly, a set of up to 16 patient-specific, somatic, single-nucleotide variants (SNVs) were selected from whole-exome sequencing of formalin-fixed, paraffin-embedded tumor tissue from the explanted liver or resected metastatic nodule and matched normal blood sample. Multiplex PCR primers were designed to target the selected SNVs and track ctDNA in the respective patients' plasma samples. Samples with ≥two SNVs above the detection confidence threshold were defined as ctDNA-positive. ctDNA levels were reported as mean tumor molecules/mL of plasma.

Statistical Analyses

The primary objective was to assess recurrence-free survival (RFS), measured between the date of LT or surgical resection and the date of radiological/clinical disease progression or HCC-related death. A secondary objective was to assess the clinical benefit of postrecurrence adjuvant therapy (AT) with regards to ctDNA dynamics. For ctDNA analysis, the molecular residual disease (MRD) window was defined as 2-12 weeks after surgery before the start of any AT. The surveillance window was defined as the post-MRD window or 2 weeks after the end of AT, and up until the last follow-up or recurrence. For ctDNA analysis at any time point after hepatectomy, patients who relapsed and did not have ctDNA test results <6 months from the date of relapse were excluded. Additionally, any postimaging ctDNA measurements were excluded. Survival analyses were performed using the Kaplan-Meier Estimator and Cox proportional hazards method. These analyses were carried out in R version 4.2.2 using packages *survminer*, *survival*, and *coxph*. All *P* values were based on two-sided testing using Wilcoxon rank-sum for unpaired analysis or Wilcoxon signed-rank for paired analysis unless specified. Comparison of image interval by ctDNA status was performed by using the linear mixed model for dependent measurements of multiple image intervals per patient using R package *lme4*. Differences were considered significant if $P \leq .05$.

RESULTS

Patient Cohorts

Of the total 125 patients included in the analyses, 93 (74.4%) were male and 32 (25.6%) were female. For the entire cohort, the median follow-up was 40 (range, 1.5-60) months. The median follow-up and disease free survival (DFS) by cohort are outlined in Table 1. Additionally, a flow chart detailing how the cohorts were separated, the presurgery and MRD ctDNA detection rate (when applicable), the median lead time, and the average number of ctDNA time points by cohort are shown in Figure 1. Clinical outcomes, duration of AT, and longitudinal ctDNA analysis for each patient in each cohort are presented in the Data Supplement (Figs S1 and S2).

ctDNA Detection in Patients With Post-Transplantation HCC (cohort A)

In cohort A, among 64 patients, who received LT, ctDNA testing was available before transplant for 21 patients and 12 were ctDNA positive. The nine ctDNA-negative patients all received neoadjuvant radiation and/or transarterial chemoembolization (TACE) before transplant. Post-transplant, 36 patients had ctDNA testing available at the MRD time point, and none of these patients were positive. All patients had serial ctDNA testing during surveillance, and one patient was found to be ctDNA positive on serial testing at 315 days post-transplant, followed by disease-related death approximately 11 months after ctDNA positivity. This led to a 100% positive predictive value. Another patient relapsed despite being serially negative. Longitudinally, among 62 nonrelapse patients with imaging available within 6 months of a ctDNA test, a specificity of 100% (62/62) and negative predictive value of 98% was observed. Taken together, ctDNA-negative patients have favorable outcomes compared with ctDNA-positive patients (hazard ratio [HR], 2.3 [95% CI, 4.0 to 10,944.0]; $P < .01$).

Association of ctDNA Status at MRD and Surveillance With Relapse-Free Survival (cohort B)

In cohort B, among 52 patients who underwent hepatectomy, 34 had ctDNA testing in the MRD time point available, and 10 (29.41%) tested positive. Of them, 10 (100%) experienced recurrence. Among the 24 MRD-negative patients, six (25.0%) experienced recurrence and two did not have outcomes available, demonstrating inferior RFS for patients with MRD positivity (HR, 7.20 [95% CI, 2.6 to 20.0]; $P < .0001$; Fig 2A). In the surveillance window, of the 36 patients with longitudinal time points, 31 patients had outcomes available. Of them, 10 (32.3%) tested positive in the surveillance window, and all 10 (100%) experienced recurrence. The remaining 21 patients (67.7%) were serially negative with two of them ultimately relapsing. Taken together, ctDNA positivity in the surveillance window was associated with a significantly poor RFS for ctDNA-positive patients

TABLE 1. Patient and Tumor Characteristics

Characteristic	N = 125
Age, median (range)	64 (20-83)
Sex, No. (%)	
Male	93 (74.4)
Female	32 (25.6)
Cohort, No. (%)	
Transplant	64 (51.2)
Hepatectomy	52 (41.6)
Postrelapse therapy	4 (3.2)
Unresectable	5 (4.0)
Overall stage, No. (%)	
I	38 (30.4)
II	51 (40.8)
III	22 (17.6)
IV	12 (9.6)
Unknown	2 (1.6)
Child-Pugh class, No. (%)	
A	55 (44.0)
B	14 (11.2)
C	4 (3.2)
Unknown	52 (41.6)
Focality, No. (%)	
Unifocal	49 (39.2)
Multifocal	45 (36.0)
Unknown	31 (24.8)
Lymphovascular invasion, No. (%)	
Absent	74 (59.2)
Present	36 (28.8)
Unknown	15 (12.0)
Histological grade, No. (%)	
Grade 1	22 (17.6)
Grade 1/2	3 (2.4)
Grade 2	52 (41.6)
Grade 2/3	3 (2.4)
Grade 3	33 (26.4)
Unknown	12 (9.6)
Etiology, No. (%)	
ALD	17 (13.6)
NAFLD/NASH	35 (28.0)
HBV	7 (5.6)
HCV	30 (24.0)
Other or NA	36 (28.8)
Adjuvant/palliative therapy,[a] No.	
IO based	
Atezolizumab + bevacizumab	4
Pembrolizumab	2
Durvalumab + tremelimumab	1
Targeted therapy: sorafenib	1
Systemic chemotherapy: gemcitabine/oxaliplatin	1
Vital status, No. (%)	
Alive	119 (95.2)
Deceased	6 (4.8)
Clinical efficacy	
Follow-up, months, median	40
DFS, months, median	
Cohort A	16 (3-52)
Cohort B	10 (1-60)
Cohort C	14 (5-15)
Cohort D (PFS)	6 (1-34)

Abbreviations: ALD, alcohol-related liver disease; DFS, disease free survival; HBV, hepatitis B virus; HCV, hepatitis C virus; IO, immuno-oncology; NA, not applicable; NAFLD/NASH, nonalcoholic fatty liver disease/nonalcoholic steatohepatitis; PFS, progression free survival.
[a]Patients who started therapy within 3 months post-transplant or surgery before any assessment of clinical recurrence by imaging were included, and inoperable cohort D patients who went to immediate systemic therapy were included.

(HR, 18.00 [95% CI, 3.9 to 85.0]; $P < .0001$; Fig 2B). Compared with patients who were serially ctDNA-negative, patients who experienced ctDNA positivity at any time point after surgery were more likely to relapse (HR, 27.00 [95% CI, 6.0 to 119.0]; $P < .0001$; Fig 2C).

ctDNA Dynamics of Individuals on Liver-Directed/Systemic Therapy (cohorts B, C, and D)

Next, we assessed whether a change in ctDNA status (ie, ctDNA-negative to ctDNA-positive) while on systemic therapy correlated with clinical benefit (defined as complete response, partial response, or stable disease). For this analysis, patients from cohorts B, C, and D were included if ctDNA testing occurred while the patient was on systemic therapy, three or more ctDNA time points were available (pre-, on-, and post-treatment), and if post-treatment imaging available (n = 12). If pre- or post-treatment time point was not available, the on-treatment time point closest to pre- and post-treatment was used. One patient, who received multiple courses of radiotherapy concurrently with different immuno-oncologic agents, was excluded from this analysis. We compared the pre- and post-treatment ctDNA test results and observed a strong correlation of ctDNA dynamics (ie, net increase or decrease) with clinical response (Fig 3A). Indeed, 100% (8/8) of patients receiving treatment showed a decrease in ctDNA and demonstrated clinical benefit from their treatment. Contrarily, 100% (4/4) of patients who showed an increase in their ctDNA had clinical progression or relapse.

Additionally, to understand how a ctDNA-positive result affected the rate of clinical imaging and time to recurrence,

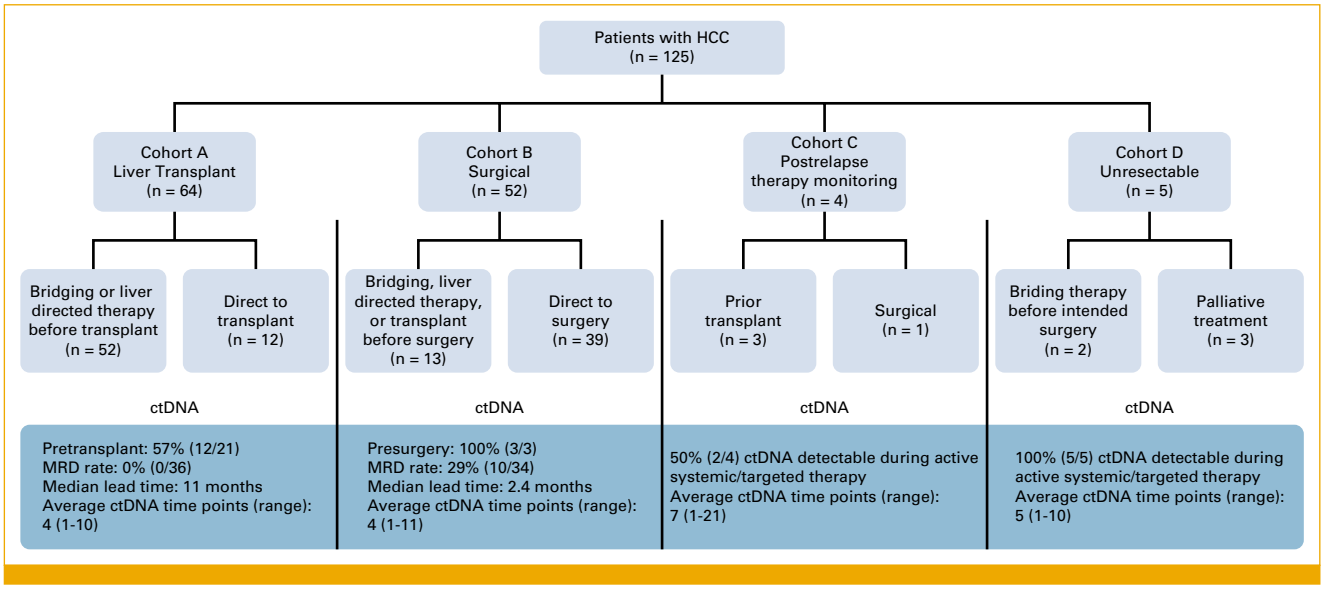

FIG 1. Flow chart detailing the number of patients in each cohort and further stratifying the clinical course and ctDNA outcomes of each cohort. ctDNA, circulating tumor DNA; HCC, hepatocellular carcinoma; MRD, molecular residual disease.

we analyzed the intervals between all imaging starting from the time of resection in cohort B. The frequency and time between imaging was evaluated based on the ctDNA status before imaging. In total, 47 patients with evaluable ctDNA and imaging results were analyzed. Of the 47 patients, 32 patients had 111 imaging results available after the ctDNA-negative result and 21 distinct patients had 110 imaging results available post-ctDNA positivity. Six patients who were initially ctDNA negative turned positive and their respective imaging studies were also analyzed according to each ctDNA group. As shown in Figure 3B, for individuals who received a ctDNA-positive result, physicians increased the frequency and shortened the average time between imaging compared with individuals who received a ctDNA-negative result (79 days [6-856] *v* 100 days [1-373], linear mixed model for dependent measurements $P = .006$). Additionally, the increased frequency of imaging led to an earlier identification of relapse in patients with ctDNA-positive results compared with patients with ctDNA-negative results. The median time to clinical recurrence by imaging after a positive ctDNA result was 59 days (4-369) versus 136 days (45-178) for ctDNA-negative patients.

Association of ctDNA Status and AFP at Anytime After Surgery With Relapse Free-Survival

Next, we directly compared the prognostic value of ctDNA and AFP. Of the 30 patients where both AFP and ctDNA testing were available, 40% (12/30) of patients experienced a recurrence and 83% (10/12) were ctDNA positive before relapse while only 42% (5/12) had elevated AFP. In these patients, despite both having an average of three tests per patient, the frequency of AFP testing was higher with a mode of four tests before relapse compared with one test for ctDNA. Additionally, the median and average time of the first ctDNA test post-hepatectomy to relapse was median: 13 months (1-35 months)/average: 13 months, compared with AFP with a median: 10 months (0-58 months)/average: 13 months. Given serial ctDNA and AFP testing occurred at different time points, a Cox regression analysis with serial ctDNA and AFP as time-dependent variables was performed to correct for the time bias. Compared with ctDNA-negative patients, ctDNA positivity was associated with a significantly shorter RFS (HR, 12.56 [95% CI, 3.70 to 42.67]; $P < .0001$). By contrast, when correcting for time bias, AFP was found to be a limited prognostic biomarker that was not statistically significant (HR, 3.67 [95% CI, 0.025 to 68.73]; $P = .48$).

We also performed a time-dependent analysis comparing the lead time between ctDNA/AFP and clinical recurrence. Of the 31 patients who had both ctDNA and AFP testing available, 11 relapsed. ctDNA significantly outperformed AFP, such that ctDNA was able to identify more recurrences and identify them significantly earlier compared with AFP ($P < .04$; Figs 4A and 4B). We found that the median lead time for ctDNA was 7.9 months before recurrence, with a maximum lead time of 16.5 months. For AFP, the median lead time was 2.2 months, with a maximum lead time of 5.5 months.

DISCUSSION

Our study highlights the potential of ctDNA testing to enhance the early detection of recurrence in patients with HCC. Serial ctDNA monitoring for early detection of relapse or disease progression has been well established in a number of solid tumors, such as colorectal, esophageal, breast, bladder, and ovarian cancers.[43-49] In HCC, previous studies have shown that preoperative and postoperative ctDNA positivity

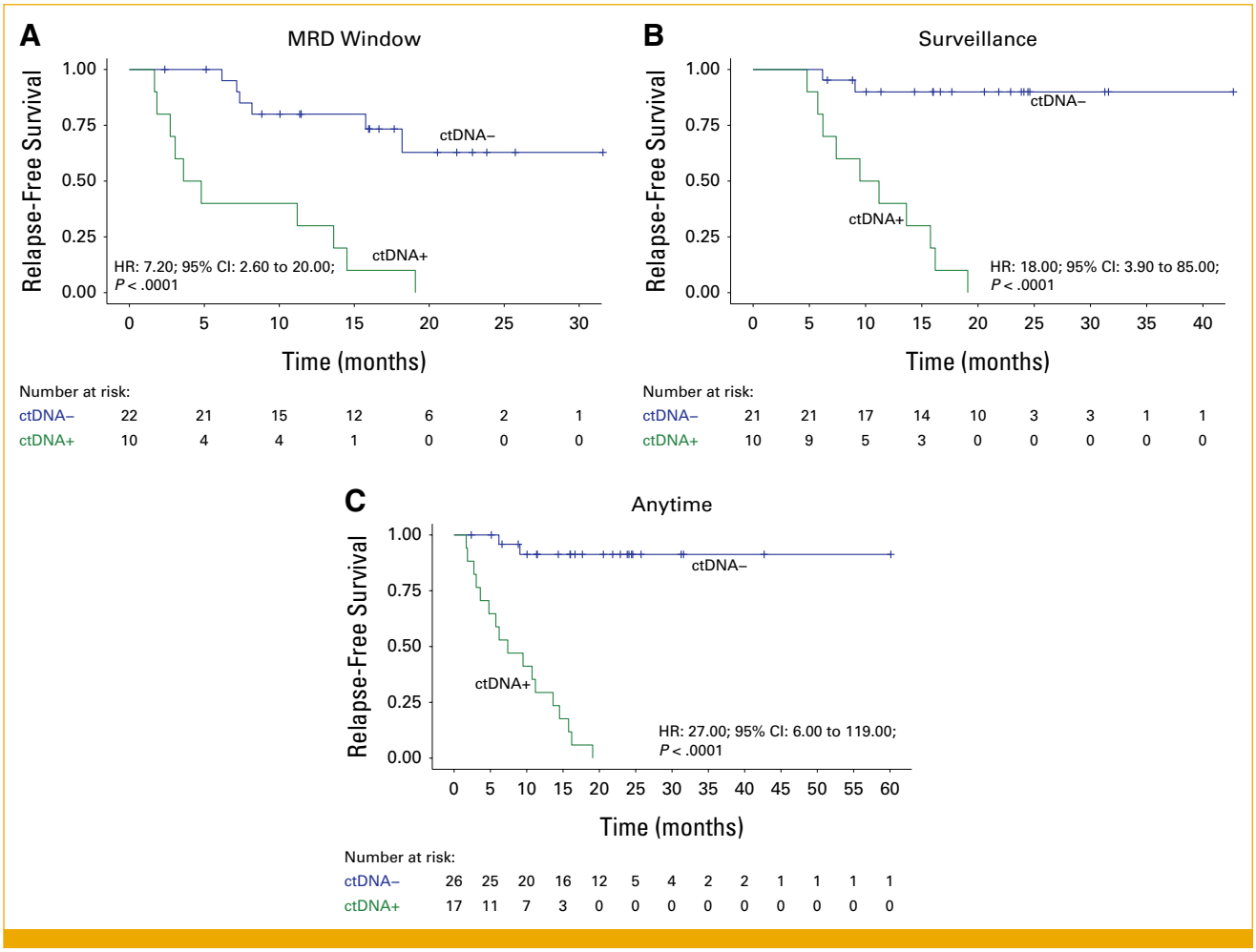

FIG 2. ctDNA status and relapse-free survival in patients with surgical HCC. (A-C) Kaplan-Meier estimates for RFS stratified by ctDNA status (ctDNA-positive, ctDNA-negative) in the (A) MRD window and (B) surveillance window. (C) Kaplan-Meier estimate for RFS on the basis of ctDNA status any time post-hepatectomy, showing that any time ctDNA positivity is prognostic of poor RFS. ctDNA, circulating tumor DNA; HCC, hepatocellular carcinoma; HR, hazard ratio; MRD, molecular residual disease; RFS, recurrence-free survival.

is associated with worse DFS and overall survival, as well as an increased risk of early tumor recurrence after liver resection.[37-39,50,51] For example, a prospective study of 285 patients with HCC showed that preoperative ctDNA detection was prognostic of early tumor recurrence postresection.[37] Despite growing evidence supporting the prognostic role of ctDNA monitoring in patients with HCC undergoing curative liver resection, very few studies have investigated the utility of ctDNA testing in patients with HCC undergoing LT.[52] Recently, however, two studies explored the utility of ctDNA testing in patients with HCC undergoing transplant.[53,54] The first study, evaluated the role of ctDNA testing for MRD detection in 74 patients with post-LT HCC. Both pre- and post-transplantation ctDNA positivity were associated with a higher rate of recurrence and shorter RFS (pretransplantation: HR, 3.25; $P = .019$; post-transplantation: HR, 4.26; $P = .010$).[53] Moreover, a decrease in ctDNA from pre- to post-transplantation was associated with favorable clinical outcomes. In the second study, pretransplantation ctDNA positivity was associated with post-transplantation recurrence (48.6% *v* 0% for ctDNA-negative patients) and a shorter DFS (390 days for ctDNA-positive *v* not reached for the ctDNA-negative; $P < .05$).[54] Of note, we observed excellent outcomes in patients who underwent LT compared with surgical resection. Despite expected differences in outcomes, current guidelines for surveillance monitoring are similar for both patient populations.[4] ctDNA offers a real-time, tumor-specific biomarker that may inform immunosuppressive modulation, which is a critical consideration in LT patients. Maintaining immunosuppression may result in increased chances of recurrence. Because adequate suppression is essential to prevent transplant rejections, a careful balance is necessary. Additionally, in transplant patients, as lifelong immunosuppression may alter immune-mediated antitumor responses, serial monitoring with ctDNA can ensure that molecular recurrence is identified early. Given that post-LT recurrence is often aggressive and time-sensitive,

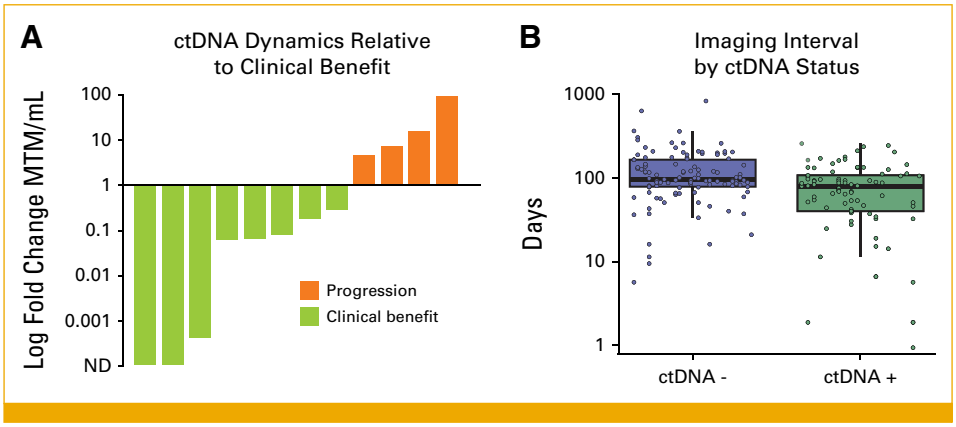

FIG 3. ctDNA dynamics are predictive of clinical benefits. (A) Waterfall plot showing the association of on-treatment ctDNA dynamics with clinical benefit/disease status among patients in cohorts B, C, and D for whom more than three ctDNA time points (pre-, on-, and post-treatment) and post-treatment imaging were available. Clinical benefit was defined as a partial response, complete response, or stable disease. Patients in green who saw a net decrease or clearance in ctDNA had a favorable clinical benefit. Patients in orange, who saw a net increase in ctDNA, progressed. (B) Clinical utility of ctDNA in the treatment of HCC. Dot plot shows individual image intervals. ctDNA testing affected the frequency of imaging. ctDNA positivity was associated with a shorter interval between imaging events. Comparison of image interval by ctDNA status was performed by using the linear mixed model for dependent measurements of multiple image intervals per patient. ctDNA, circulating tumor DNA; HCC, hepatocellular carcinoma; MTM, mean tumor molecules; ND, not determined.

ctDNA monitoring provides a unique opportunity to optimize surveillance and guide therapeutic decision making. These findings support further investigation in larger prospective studies to refine the clinical utility of ctDNA in this distinct population.

Furthermore, we demonstrate that ctDNA monitoring provides insights into treatment response in HCC. Specifically, changes in ctDNA levels during treatment correlated with clinical outcomes, suggesting its utility as a biomarker for assessing therapy effectiveness. This finding aligns with

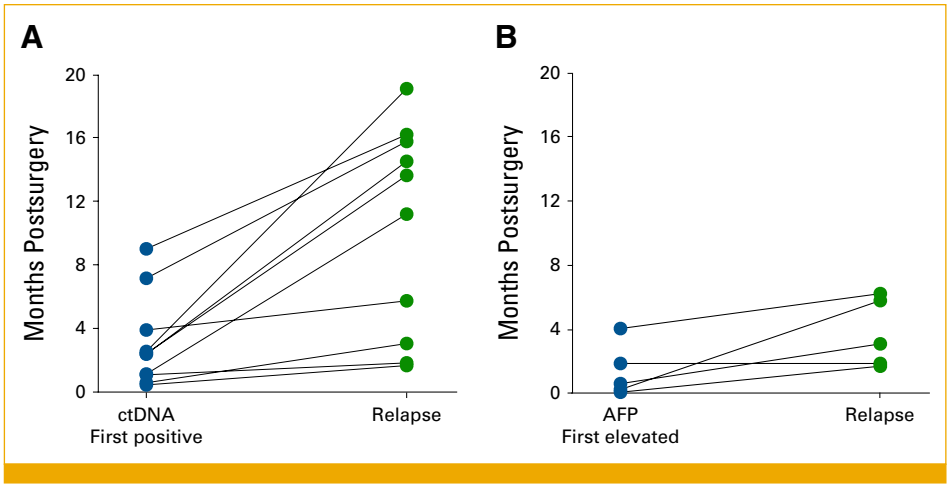

FIG 4. ctDNA-based MRD testing is predictive of relapse-free survival in patients with HCC, a similar outcome was found with AFP testing. (A) ctDNA-based MRD testing was capable of detecting relapse in patients with HCC months earlier than AFP testing. Time to relapse after the first ctDNA-positive test had a median lead time of 7.9 months, with a maximum lead time of 16.5 months. (B) Time to relapse after the first elevated AFP test had a median lead time of 2.2 months, with a maximum lead time of 5.5 months. ctDNA was able to identify more recurrences and identify them significantly earlier compared with AFP ($P < .04$). *P* value was obtained using the Wilcoxon signed-rank test. AFP, alpha-fetoprotein; ctDNA, circulating tumor DNA; HCC, hepatocellular carcinoma; MRD, molecular residual disease.

previous studies that have evaluated the role of ctDNA dynamics in monitoring treatment responses across various cancers, including HCC. For example, one study reported that tumor-informed ctDNA analysis during systemic therapy in patients with HCC helped identify early signs of tumor response or progression, enabling timely adjustments to treatment plans.[55] Similarly, another demonstrated ctDNA clearance during therapy was associated with improved progression free survival, whereas persistent ctDNA detection predicted poorer outcomes in patients with HCC.[56] These findings suggest that ctDNA dynamics can complement radiographic assessments by offering a more sensitive, real-time indication of tumor behavior.

In our cohort, ctDNA monitoring was evaluated in patients across multiple clinical scenarios, including surveillance after curative-intent surgery or transplantation, treatment monitoring in patients with recurrent disease, and systemic therapy in those with inoperable, advanced-stage HCC. Although some analyses combined these groups, we presented cohorts C and D separately to reflect key clinical distinctions: patients in cohort C experienced recurrence after prior curative-intent treatment, whereas patients in cohort D were inoperable at diagnosis and received systemic therapy as their initial treatment. Importantly, although ctDNA was used for treatment monitoring in both cohorts, clinical utility differed. For example, in cohort C, ctDNA could be used to monitor patients closely and assess therapeutic response to identify opportunities for further curative intervention, including treatment escalation or modification. By contrast, in cohort D, where patients had no curative options, ctDNA dynamics could play a role in evaluating disease trajectory and guiding palliative care decisions. As such, even subtle differences in ctDNA trends might be interpreted differently between cohorts. These differences underscore the importance of clinical context in interpreting ctDNA results and support its use as a dynamic, individualized decision-making tool across diverse populations with HCC. Additionally, previous studies did not incorporate ctDNA testing in the surveillance setting, and, in our study, we demonstrated the feasibility of using a personalized, tumor-informed ctDNA assay for longitudinal monitoring in a post-transplantation surveillance setting in patients with HCC. Together, our results and existing literature support the integration of ctDNA monitoring into clinical workflows as a dynamic biomarker for evaluating treatment efficacy.

AFP is the most widely used blood-based biomarker used during screening for HCC and post-treatment surveillance in patients with HCC. However, multiple studies have reported that AFP shows poor sensitivity and specificity.[20,28] Additionally, elevated AFP levels can also be observed during pregnancy, chronic hepatitis, cirrhosis, and other malignancies such as intrahepatic cholangiocarcinoma, metastatic colon cancer, and germ cell tumors.[57-60] Falsely elevated AFP in the setting of hepatitis/cirrhosis is challenging because most HCC cases develop in the setting of those conditions, making it difficult to differentiate whether elevated AFP indicates cancer progression or worsening underlying liver disease. Furthermore, AFP levels within normal limits have been reported in 30%-40% of patients with HCC.[59-61] In our cohort, pretransplantation AFP measurements were available for 35 patients, 28 of whom had normal AFP levels. And while AFP testing is currently integrated into standard practice, ctDNA outperforms AFP. In our cohort, despite the differences in the median time from first test to relapse, the average time of first test to relapse was similar at 13 months. Despite the greater cadence of AFP testing, ctDNA identified more relapse cases and detected recurrences earlier (7.9 *v* 2.2 months). Although the exact lead time improvement over AFP cannot be determined given the differences in timing of AFP and ctDNA testing, the data strongly suggest that ctDNA measurements in conjunction with AFP testing may lead to improved clinical management for patients with HCC.

Importantly, ctDNA detection predicted recurrence on average 7.9 months ahead of radiographic relapse. A multi-institutional study has shown that early detection of relapse through increased surveillance imaging can lead to aggressive/potentially curative treatment options and result in improved postrecurrence survival.[23] The authors concluded that three radiographic scans in the first 24 months after curative-intent surgery are associated with the ability to offer effective treatment options. This underscores the wide variability in outcomes among individuals with HCC and the need for a more personalized surveillance approach. In our data set, we observed that ctDNA-based MRD detection can prompt radiographic imaging earlier than routine surveillance imaging per current clinical practice, which may offer additional opportunities to treat isolated recurrences.

On the other hand, ctDNA negativity during post-transplantation monitoring could help reduce imaging frequency as aggressive surveillance imaging is unnecessary. As demonstrated in our study, a cohort of 62 post-transplant patients were ctDNA-negative and remained relapse-free, yielding a specificity of 100%. ctDNA-based MRD surveillance monitoring addresses one of the unmet clinical needs determined by a report from the ILTS, which stated a need for a system to identify patients with a minimal risk of HCC recurrence in whom surveillance may not be recommended.[25] Thus, the high specificity demonstrated here holds a clinical relevance for transplant clinicians enabling them to make an informed decision to reliably offer less intensive surveillance regimens. Taken together, ctDNA has important clinical utility in patients with HCC.

This study provides real-world data on the use of ctDNA testing in patients with HCC. Although several limitations exist, namely a relatively small cohort size, variability in ctDNA time points, differences in treatment context, and variations in surgical and pretransplant/surgical AT

received, the data demonstrate the potential of longitudinal, personalized, tumor-informed ctDNA monitoring as a tool in patients with curatively treated HCC during surveillance. Additional larger, prospective studies are needed to further validate the clinical utility of ctDNA-based surveillance testing in HCC, including the addition of specific experimental treatments on the basis of molecular recurrence.

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