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# Circulating Tumor DNA Profiling in Liver Transplant for Hepatocellular Carcinoma, Cholangiocarcinoma, and Colorectal Liver Metastases: A Programmatic Proof of Concept

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**Simple Summary:** Circulating tumor DNA (ctDNA) is emerging as a diagnostic and surveillance tool in cancer and recurrence. The recurrence rates after liver transplant for cancer are significant, highlighting the need for early detection and treatment. We report a cohort of patients who underwent liver transplant for hepatocellular carcinoma, cholangiocarcinoma, or colorectal cancer liver metastasis and received ctDNA testing pre- and/or post-transplant. We aim to show how ctDNA testing can be incorporated into pre-transplant work-up and post-transplant surveillance and discuss the benefits of this testing modality in the identification of genetic targets and surveillance of recurrence.

**Abstract:** Introduction: Circulating tumor DNA (ctDNA) is emerging as a promising, non-invasive diagnostic and surveillance biomarker in solid organ malignancy. However, its utility before and after liver transplant (LT) for patients with primary and secondary liver cancers is still underexplored. Methods: Patients undergoing LT for hepatocellular carcinoma (HCC), cholangiocarcinoma (CCA), and colorectal liver metastases (CRLM) with ctDNA testing were included. CtDNA testing was conducted pre-transplant, post-transplant, or both (sequential) from 11/2019 to 09/2023 using Guardant360, Guardant Reveal, and Guardant360 CDx. Results: 21 patients with HCC ($n$ = 9, 43%), CRLM ($n$ = 8, 38%), CCA ($n$ = 3, 14%), and mixed HCC/CCA ($n$ = 1, 5%) were included in the study. The median follow-up time was 15 months (range: 1–124). The median time from pre-operative testing to surgery was 3 months (IQR: 1–4; range: 0–5), and from surgery to post-operative testing, it was 9 months (IQR: 2–22; range: 0.4–112). A total of 13 (62%) patients had pre-transplant testing, with 8 (62%) having ctDNA detected (ctDNA+) and 5 (32%) not having ctDNA detected (ctDNA-). A total of 18 (86%) patients had post-transplant testing, 11 (61%) of whom were ctDNA+ and 7 (33%) of whom were ctDNA-. The absolute recurrence rates were 50% ($n$ = 5) in those who were ctDNA+ vs. 25% ($n$ = 1) in those who were ctDNA- in the post-transplant setting, though this difference was not statistically significant ($p$ = 0.367). Six (29%) patients (HCC = 3, CCA = 1, CRLM = 2) experienced recurrence with a median recurrence-free survival of 14 (IQR: 6–40) months. Four of these patients had positive post-transplant ctDNA collected following diagnosis of recurrence, while one patient had positive post-transplant ctDNA collected preceding recurrence. A total of 10 (48%) patients had sequential ctDNA testing, of whom $n$ = 5 (50%) achieved ctDNA clearance (+/−). The remainder were ctDNA+/+ ($n$ = 3, 30%), ctDNA−/− ($n$ = 1, 10%), and ctDNA−/+ ($n$ = 1, 11%). Three (30%) patients

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showed the acquisition of new genomic alterations following transplant, all without recurrence. Overall, the median tumor mutation burden (TMB) decreased from 1.23 mut/Mb pre-transplant to 0.00 mut/Mb post-transplant. Conclusions: Patients with ctDNA positivity experienced recurrence at a higher rate than the ctDNA- patients, indicating the potential role of ctDNA in predicting recurrence after curative-intent transplant. Based on sequential testing, LT has the potential to clear ctDNA, demonstrating the capability of LT in the treatment of systemic disease. Transplant providers should be aware of the potential of donor-derived cell-free DNA and improved approaches are necessary to address such concerns.

**Keywords:** liver transplant; circulating tumor DNA; liquid biopsy; liver cancer; hepatocellular carcinoma; cholangiocarcinoma; colorectal liver metastasis

## 1. Introduction

Liver transplant is the only curative-intent treatment option for patients with unresectable hepatocellular carcinoma, cholangiocarcinoma, and colorectal liver metastasis [1–6]. However, the recurrence rates may be as high as 20–50% in certain conditions, highlighting the need for thorough and frequent post-transplant monitoring [3,4,7–9]. Traditional surveillance relies on cross-sectional imaging and serum biomarkers such as alpha-fetoprotein (AFP), carbohydrate/cancer antigen 19-9 (CA19-9), or carcinoembryonic antigen (CEA) depending on the disease. However, post-transplant recurrence remains a challenge in terms of diagnostics and treatment due to the limited sensitivity and specificity of current tools for cancer detection [10–14].

To address this issue, ctDNA-based liquid biopsy has emerged as a non-invasive approach that allows for the real-time monitoring of tumor dynamics, detection of minimal residual disease, and identification of actionable mutations [15–19]. In patients undergoing liver transplant, the use of cell-free DNA has also been applied to detecting rejection [20]. We have previously reported the use of ctDNA in patients undergoing liver transplant for CRLM [21]. However, its use as a predictive tool of recurrence in liver transplant remains to be fully explored.

Herein, we present a cohort of patients who underwent liver transplant for HCC, CCA, or CRLM and ctDNA testing at pre-transplant and/or post-transplant time points, demonstrating a proof of concept for ctDNA in this setting.

## 2. Methods

Patients who underwent liver transplantation for CRLM, HCC, or CCA with pre-transplant and/or post-transplant ctDNA assessment between November 2019 and September 2023 at a single quaternary care academic institution were included in this study. All the patients were evaluated by a multidisciplinary liver tumor board and liver transplant review committee. The demographic and clinical variables, including on imaging, laboratory values, and treatment courses, were collected via a retrospective review of the patients' health charts, as approved by the Institutional Review Board (IRB).

The ctDNA was assessed using Guardant360, Guardant360 CDx, and Guardant Reveal assays (Guardant Health, Redwood City, CA, USA). Guardant360 uses next-generation sequencing (NGS) to detect clinically relevant genomic alterations in the circulating tumor DNA in plasma collected via the peripheral blood. NGS testing was performed as part of the standard clinical care in a CLIA-certified and College of American Pathologists-accredited laboratory. The blood was collected in two to four 10 mL Streck tubes, and the processed plasma was evaluated for single-nucleotide variants (SNVs), insertions–deletions (indels), gene fusions/rearrangements, and copy number variants (CNVs) across 83 genes. Mutations were annotated using OncoKB to define pathogenic variants. The blood tumor mutational burden (bTMB) was determined by analyzing the somatic SNVs and indels across a 1.0 Mb genomic backbone. For the TMB algorithm, common cancer drivers and

resistance alterations, as well as putative CHIP alterations, were filtered from the analysis. Guardant Reveal uses NGS to determine the presence of ctDNA by assessing somatic alterations (SNVs, insertion–deletion alterations) and epigenomic signatures (methylation status). Guardant Reveal was used for the portion of patients with CRLM, while Guardant360 CDx was used for the portion of patients with HCC. Guardant360 was used for all cancer types.

Prior to January 2021, the ctDNA was collected and evaluated at the discretion of the treating surgeon. From January 2021 onward, attempts were made to collect the ctDNA at times outlined by the current institutional protocol of within 30 days pre-operatively, 30–60 days post-operatively, and every 3–6 months afterward (Figure 1). Synonymous mutations were excluded from the analysis.

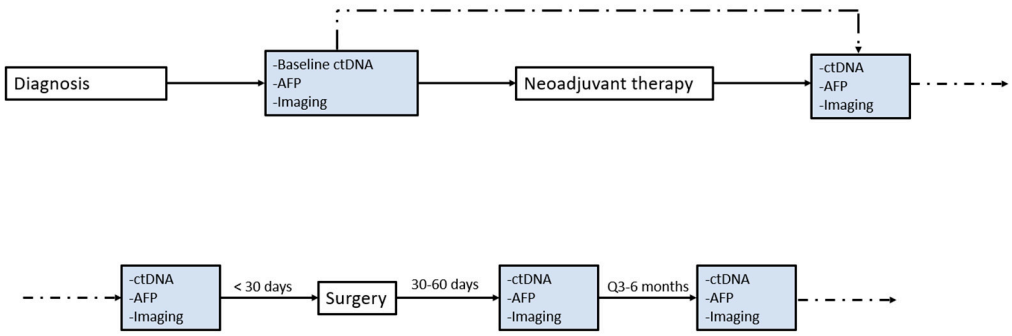


**Figure 1.** Timeline for ctDNA testing, cancer work-up, and surveillance as per institutional protocol. Note the example tumor marker shown is AFP for HCC; for other cancer types, the corresponding serum tumor marker (CCA: CA19-9, CRLM: CA19-9) is used for assessment.

Discrete variables were presented as frequency and percentages, and continuous variables were presented as medians with interquartile ranges due to non-normal distributions. Statistical analysis was performed using IBM SPSS Statistics Version 29.0 (Armonk, New York, NY, USA). A two-sided $p$-value < 0.05 was considered significant for all tests.

## 3. Results

A total of 21 patients underwent ctDNA testing and LT for HCC ($n$ = 9, 43%), CRLM ($n$ = 8, 38%), CCA ($n$ = 3, 14%), and mixed HCC/CCA ($n$ = 1, 5%) (Table 1). Nine (43%) patients underwent living donor liver transplant (LDLT), seven (33%) underwent orthotopic liver transplant (OLT) with grafts from donation after brain death (DBD), and five (24%) had OLT with grafts from donation after cardiac death (DCD) (Table 1). Most patients had cirrhosis ($n$ = 19, 90%), with a median MELD score of 15 at the time of transplant. NASH ($n$ = 6, 30%) was the most frequent cause of cirrhosis. The median tumor marker levels at the time of liver cancer diagnosis were AFP = 8 ng/mL (HCC), CA19-9 = 23 U/mL (CCA), and CEA = 31 ng/mL (CRLM). (Table 1). Prior to transplant, most patients ($n$ = 18, 86%) received treatment, the most common being chemotherapy ($n$ = 10, 48%), radiation ($n$ = 6, 29%), TACE ($n$ = 6, 29%), and TARE ($n$ = 5, 24%). The post-transplant tumor marker levels were 3 ng/mL, 13 U/mL, and 1.8 ng/mL for AFP, CA19-9, and CEA, respectively (Table 1). Six (29%) patients (HCC = 3, CCA = 1, CRLM = 2) experienced recurrence with a median recurrence-free survival of 14 (IQR: 6-40) months. Two (10%) patients experienced cancer-related death, both with a diagnosis of HCC (Table 1). Overall, four (19%) patients experienced mortality, with a median overall survival of 16 (IQR: 8–40) months. The median and maximum follow-up times were 15 and 124 months, respectively (Table 1).

**Table 1.** Summary of demographic and pre-transplant variables and post-transplant outcomes.

| | **ALL N = 21** | **HCC N = 9** | **HCC/CCA N = 1** | **CCA N = 3** | **CRLM N = 8** |
|---|---|---|---|---|---|
| Male Sex, N (%) | 16 (76%) | 8 (89%) | 0 | 2 (67%) | 6 (75%) |
| Race, N (%) | | | | | |
| White | 18 (86%) | 8 (89%) | 1 (100%) | 2 (50%) | 7 (88%) |
| Black | 2 (10%) | 0 | 0 | 1 (25%) | 1 (13%) |
| Other/Unknown | 1 (5%) | 1 (11%) | 0 | 0 | 0 |
| Age at Transplant Surgery, Median (IQR) | 55 (50–68) | 70 (46–73) | 60 | 51 (25–55) | 54 (49–60) |
| Cirrhosis, N (%) | 19 (90%) | 9 (100%) | 1 (100%) | 3 (75%) | 6 (86%) |
| Non-Malignancy Cirrhosis Factors | | | | | |
| A1AT | 1 (5%) | 0 | 1 (100%) | 0 | 0 |
| ETOH | 2 (10%) | 1 (11%) | 0 | 1 (25%) | 0 |
| HBV | 3 (14%) | 2 (22%) | 1 (100%) | 0 | 0 |
| HCV | 1 (5%) | 1 (11%) | 0 | 0 | 0 |
| NASH | 6 (29%) | 5 (56%) | 0 | 0 | 1 (14%) |
| PSC | 2 (10%) | 0 | 0 | 1 (25%) | 1 (14%) |
| Biliary Atresia | 2 (10%) | 2 (22%) | 0 | 0 | 0 |
| Chemotherapy-Induced | 1 (5%) | 0 | 0 | 0 | 1 (14%) |
| PBC | 1 (5%) | 0 | 0 | 0 | 1 (14%) |
| MELD Score, Median (IQR) | 15 (11–24) | 22 (14–25) | 24 | 12 (10–29) | 11 (7–19) |
| Pre-Treatment Tumor Marker Level, Mean (SD) | | | | | |
| AFP (ng/mL) | 8 (6, 14) | 8 (6, 13) | 39 | | |
| CA19-9 (U/mL) | 23 (12, 168) | | 216 | 22 (8, 24) | |
| CEA (ng/mL) | 31 (1, 64) | | | | 31 (1, 64) |
| Pre-Transplant Number of Lesions, N (%) | | | | | |
| 1 | 11 (52%) | 7 (78%) | 0 | 4 (100%) | 0 |
| 2–3 | 6 (29%) | 1 (11%) | 1 (100%) | 0 | 4 (50%) |
| Innumerable | 2 (10%) | 0 | 0 | 0 | 2 (25%) |
| Pre-Transplant Size of Biggest Lesion (cm), Median (IQR) | 4 (2–6) | 1 (1–4) | 3 | 1 | 6.7 (3–8) |
| Pre-Transplant Treatment, N (%) | 18 (86%) | 7 (78%) | 0 | 3 (100%) | 8 (100%) |
| Systemic Chemotherapy | 10 (48%) | 0 | 0 | 2 (67%) | 8 (100%) |
| Radiotherapy | 6 (29%%) | 0 | 0 | 2 (67%) | 4 (50%) |
| SBRT | 4 (19%) | 0 | 0 | 2 (67%) | 2 (25%) |
| Prior Surgery | 3 (14%) | 0 | 0 | 0 | 3 (38%) |
| Ablation | 4 (19%) | 1 (11%) | 0 | 0 | 3 (38%) |
| Chemoembolization | 6 (29%) | 2 (22%) | 0 | 0 | 4 (50%) |
| Radioembolization | 5 (24%) | 4 (44%) | 0 | 0 | 1 (13%) |
| Immunotherapy | 1 (5%) | 0 | 0 | 0 | 1 (13%) |
| Post-Transplant Tumor Marker Level, Median (IQR) | | | | | |
| AFP (ng/mL) | 3 (3–7) | 3 (3–6.8) | 4.8 | | |
| CA19-9 (U/mL) | 13 (6–20) | | | 13 (6–20) | |
| CEA (ng/mL) | 2 (1–2) | | | | 1.7 (1–2) |
| Recurrence, N (%) | 6 (29%) | 3 (33%) | 0 | 1 (25%) | 2 (25%) |
| Patient Status, N (%) | | | | | |
| Alive | 17 (81%) | 6 (67%) | 1 (100%) | 2 (67%) | 8 (100%) |
| Dead | 4 (19%) | 3 (33%) | 0 | 1 (33%) | 0 |
| Cancer-Related Deaths, N (%) | 2 (10%) | 2 (22%) | 0 | 0 | 0 |
| Recurrence Survival (Days), Median (IQR) | 13 (7–28) | 12 (5–31) | 16.7 | 8 (6–15) | 14 (10–40) |
| Overall Survival (Days), Median (IQR) | 14 (8–39) | 14 (6–34) | 16.7 | 8 (6–32) | 25 (10–60) |

Key: A1AT = alpha-1-anti-trypsin deficiency, ETOH = ethanol, HBV = hepatitis B virus, HCV = hepatitis C virus, NASH = non-alcoholic steatohepatitis, PSC = primary sclerosing cholangitis, PBC = primary biliary cholangitis, AFP = alpha-fetoprotein, CA19-9 = cancer antigen 19-9, CEA = carcinoembryonic antigen, SBRT = stereotactic body radiation therapy.

In terms of transplant, most patients ($n$ = 18, 86%) underwent the piggyback technique (Table 2). The median warm ischemia time was 43 (IQR: 39–46) minutes, with a median LT duration of 589 (IQR: 471–702) minutes. Post-operatively, most patients underwent induction immunosuppression with basiliximab ($n$ = 12, 57%) and initial immunosuppression with glucocorticoids, mycophenolate mofetil, and tacrolimus ($n$ = 19, 90%) (Table 3). Five (24%) patients experienced bile leaks, requiring ERCP and/or PTHC, while three (14%) patients experienced biliary strictures requiring ERCP (Table 3). One patient had ischemic cholangiopathy and hepatic artery stenosis in addition to their biliary leak, requiring HJ reconstruction, PTHC, re-transplant, and stent placement (Table 3). Two patients experienced mild acute rejection, which was treated with IV steroids. No patients experienced chronic rejection (Table 3).

A total of 18 (86%) patients had post-transplant ctDNA, with 11 having ctDNA detected and 7 not having ctDNA detected (Table 4). The absolute recurrence rates were higher in patients with detected ctDNA ($n$ = 5, 50%) compared to patients without ctDNA detected ($n$ = 1, 25%), although this difference was not found to be statistically significant ($p$ = 0.367).

Of the six (29%) patients with recurrence, five patients had post-transplant ctDNA detected. The remaining patient (#20) did not have post-transplant ctDNA detected during or after treatment of their recurrence with metastasectomy and chemotherapy (Tables 4 and 5). Of the post-transplant ctDNA+ patients, 4/5 had ctDNA detected following radiologic detection of recurrence, while 1/5 (#7) had ctDNA detected prior to recurrence, without elevated tumor markers. Overall, only 3/6 patients (#3, 12, 21) had elevated serum tumor markers preceding recurrence, while 3/6 (#6, 7, 20) patients lacked elevation of the traditionally used tumor markers prior to recurrence.

Of all 21 patients, 10 (48%) patients had sequential ctDNA testing, with half ($n$ = 5, 50%) having ctDNA clearance (+/−). The remainder were ctDNA+/+ ($n$ = 3, 30%), ctDNA−/− ($n$ = 1, 10%), and ctDNA−/+ ($n$ = 1, 10%). More specifically, patients #9, 11, 15, 16, and 18 were ctDNA+/−; patients #2, 17, and 21 were ctDNA +/+; patient #13 was ctDNA−/−; and patient #14 was ctDNA−/+. Of note, patient #21 experienced recurrence. Three (30%) patients showed the acquisition of new genomic alterations in post-transplant ctDNA (#2, 14, 17) (Table 6). Patients #14 and 17 had no evidence of histopathologic viable tumors on explant (Table 7), suggesting potential alternate sources of these ctDNA findings. No signs of acute rejection were noticed for these patients (#14, #17, Table 6).

Overall, the median TMB decreased from 1.23 mut/Mb pre-transplant ($n$ = 9) to 0.00 mut/Mb post-transplant ($n$ = 11). For HCC, a *TERT* promoter mutation was the most common genomic alteration both pre-transplant and post-transplant (Figure 2). For CRLM, *TP53* and *APC* mutations were the most common alterations observed pre-transplant, compared to *NF1* and *PTPN11* post-transplant (Figure 2).

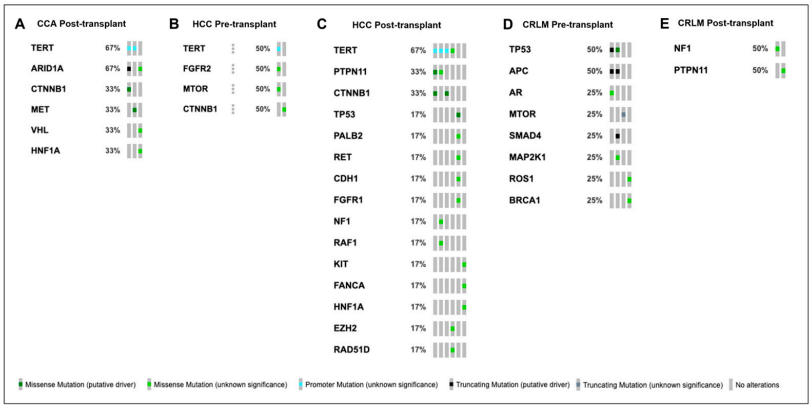


**Figure 2.** Oncoprints of genomic alterations detected using Guardant 360 available for CCA post-transplant (**A**), HCC pre-transplant (**B**), HCC post-transplant (**C**), CRLM pre-transplant (**D**), and CRLM post-transplant (**E**). Type of genomic alteration represented by color with key at bottom of figure.

**Table 2.** Transplant variables.

| Pt | Age | Sex | Cancer Type | Cirrhosis Factors | MELD at Tx | Tx Type | Liver Transplant Technique | Aberrant Liver Vasculature | Type of Arterial Anastomosis | Type of Venous Anastomosis | Biliary Anastomosis | Real Warm Ischemia Time (min) | LT Duration (min) | RBCs, FFP (Units) | Reperfusion Order | Post-Reperfusion Syndrome |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **1** | 39 | M | HCC | Biliary atresia, PBC | 12 | LDLT | Piggyback | - | Standard | Interposition | HJ | 38 | 776 | 0, 0 | Vein first | No |
| **2** | 70 | M | HCC | NASH | 22 | DCD | Piggyback | - | Standard | End-to-end | Duct-to-duct | 43 | 541 | 6, 3 | Vein first | No |
| **3** | 52 | M | HCC | HCV | 23 | DCD | Conventional | Replaced RHA | Standard | End-to-end | Duct-to-duct | - | - | - | Vein first | Yes |
| **4** | 75 | M | HCC | NASH | 25 | DBD | Piggyback | - | Standard | End-to-end | Duct-to-duct | 46 | 505 | 2, 0 | Vein first | No |
| **5** | 66 | M | HCC | NASH, ETOH, HBV | 9 | LDLT | Piggyback | Accessory LHA | Standard | End-to-end | Duct-to-duct | 39 | 720 | 0, 0 | Vein first | No |
| **6** | 70 | M | HCC | HBV | 25 | DBD | Piggyback | Accessory RHA | Standard | End-to-end | Duct-to-duct | 46 | 430 | 0, 0 | Vein first | No |
| **7** | 72 | M | HCC | NASH | 11 | LDLT | Piggyback | - | Standard | End-to-end | Duct-to-duct | 42 | 557 | 7, 5 | Vein first | No |
| **8** | 73 | M | HCC | NASH | 12 | DCD | Piggyback | - | Standard | End-to-end | Duct-to-duct | 58 | 410 | 4, 1 | Both | No |
| **9** | 32 | F | HCC | Biliary atresia | 40 | Split, DBD | Conventional | - | Standard | End-to-end | HJ | 45 | 657 | 18, 13 | Vein first | No |
| **10** | 60 | F | HCC/CCA | HBV, A1AT | 24 | DCD | Piggyback | - | Standard | End-to-end | Duct-to-duct | 48 | 385 | 8, 1 | Both | Yes |
| **11** | 25 | M | CCA | PSC | 12 | DCD | Conventional | - | Standard | End-to-end | HJ | 42 | 490 | 3, 0 | Vein first | Yes |
| **12** | 51 | F | CCA | ETOH | 29 | DBD | Piggyback | Replaced LHA | Standard | Conduit | HJ | 27 | 683 | 17, 11 | Vein first | Yes |
| **13** | 55 | M | CCA | - | 10 | DBD | Conventional | Replaced RHA | Infra-renal | Conduit | HJ | 40 | 452 | 1, 0 | Vein first | No |
| **14** | 50 | M | CRLM | - | 6 | LDLT | Piggyback | - | Standard | End-to-end | Duct-to-duct | 39 | 584 | 0, 0 | Vein first | No |
| **15** | 53 | M | CRLM | - | 6 | DBD | Conventional | - | Standard | End-to-end | HJ | 49 | 427 | 0, 0 | Vein first | Yes |
| **16** | 61 | M | CRLM | - | 13 | LDLT | Conventional | - | Standard | End-to-end | Duct-to-duct | 32 | 869 | 4, 0 | Vein first | No |
| **17** | 64 | M | CRLM | - | 11 | LDLT | Piggyback | - | Standard | End-to-end | Duct-to-duct | 43 | 594 | 7, 8 | Vein first | No |
| **18** | 54 | M | CRLM | - | 14 | LDLT | Piggyback | - | Standard | Interposition | Duct-to-duct | 67 | 992 | 5, 0 | Vein first | No |
| **19** | 49 | F | CRLM | PBC | 23 | LDLT | Piggyback | - | Standard | End-to-end | Duct-to-duct | 27 | 685 | 2, 0 | Vein first | No |
| **20** | 49 | M | CRLM | - | 21 | DBD | Piggyback | - | Standard | End-to-end | HJ | 39 | 708 | 20, 12 | Vein first | No |
| **21** | 56 | F | CRLM | NASH | 8 | LDLT | Piggyback | - | Standard | Interposition | Duct-to-duct | 45 | 700 | 4, 0 | Vein first | Yes |

Key: PBC = primary biliary cholangitis, NASH = non-alcoholic steatohepatitis, HCV = hepatitis C virus, ETOH = ethanol, HBV = hepatitis B virus, A1AT = alpha-1 anti-trypsin deficiency, PSC = primary sclerosing cholangitis, LDLT = living donor liver transplant, DCD = donation after cardiac death, DBD = donation after brain death, RHA = right hepatic artery, LHA = left hepatic artery, HJ = hepaticojejunostomy.

**Table 3.** Post-transplant variables.

| Patient | Induction IS | Initial IS | IS 12 month | Biliary Complications | Biliary Intervention | Arterial Complications, Intervention | Acute Rejection Grade | Treatment of Acute Rejection | Chronic Rejection |
|---|---|---|---|---|---|---|---|---|---|
| **1** | Basiliximab | GC + Tacrolimus | - | Leak | PTHC | - | - | - | - |
| **2** | - | GC + MMF + Tacrolimus | - | - | - | - | - | - | - |
| **3** | Basiliximab | GC + Tacrolimus | Tacrolimus + Sirolimus | - | - | - | - | - | - |
| **4** | Basiliximab | GC + MMF + Tacrolimus | Cyclosporine + Everolimus | - | - | - | - | - | - |
| **5** | Basiliximab | GC + MMF + Tacrolimus | Tacrolimus + MMF | Leak | ERCP | - | Mild | IV steroids | - |
| **6** | Basiliximab | GC + MMF + Tacrolimus | Tacrolimus + MMF + Sirolimus | - | - | - | - | - | - |
| **7** | Basiliximab | GC + MMF + Tacrolimus | - | - | - | - | - | - | - |
| **8** | - | GC + MMF + Tacrolimus | Tacrolimus + Everolimus | Stricture | ERCP | - | - | - | - |
| **9** | - | GC + MMF + Tacrolimus | - | - | - | - | - | - | - |
| **10** | Basiliximab | GC + MMF + Tacrolimus | Tacrolimus + Everolimus | - | - | - | - | - | - |
| **11** | - | GC + MMF + Tacrolimus | - | Leak, ischemic cholangiopathy | HJ reconstruction, PTHC, re-transplant | HA stenosis and pseudoaneurysm, stent placement | - | - | - |
| **12** | Basiliximab | GC + MMF + Tacrolimus | Tacrolimus + GC + MMF | - | - | - | - | - | - |
| **13** | - | GC + MMF + Tacrolimus | - | - | - | - | - | - | - |
| **14** | Basiliximab | GC + MMF + Tacrolimus | - | - | - | - | - | - | - |
| **15** | - | GC + MMF + Tacrolimus | Tacrolimus + Everolimus | Leak | Re-operation | - | - | - | - |
| **16** | Basiliximab | GC + MMF + Tacrolimus | - | Stricture | ERCP | - | Mild | IV steroids | - |
| **17** | Basiliximab | GC + MMF + Tacrolimus | Tacrolimus + Everolimus | Leak | ERCP + PTC | - | - | - | - |
| **18** | Basiliximab | GC + MMF + Tacrolimus | Tacrolimus + Everolimus | Stricture | ERCP | - | - | - | - |
| **19** | - | GC + MMF + Tacrolimus | - | - | - | - | - | - | - |
| **20** | - | GC + MMF + Tacrolimus | - | - | - | - | - | - | - |
| **21** | - | GC + MMF + Tacrolimus | - | - | - | - | - | - | - |

**Table 4.** Tumor marker correlation with ctDNA testing at times prior to and following transplant, along with time of recurrence.

| Pt | Cancer Type | Date Liver Cancer dx | Dx Date Tumor Marker Level | Dx Tumor Marker Level | Date Pre-Transplant Marker | Pre-Transplant Tumor Marker | Date of Pre-Transplant ctDNA | Pre- ctDNA Results | Date of Transplant | Date Post-Transplant Marker | Post-Transplant Tumor Marker | Date of Post-Transplant ctDNA | Post-Transplant ctDNA Results | ctDNA Timing | Date of Recurrence | Date of Tumor Marker Level with Recurrence | Recurrence Tumor Marker Level |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **1** | HCC | 7/2022 | 8/2/2023 | AFP: 15 | 8/15/23 | AFP: 24.1 | 8/15/23 | + (CDx) | 8/21/23 | 10/26/23 | AFP: <3.0 | | | | | | |
| **2** | HCC | 2/16/2023 | 4/17/2023 | AFP: <3 | 4/17/2023 | AFP: <3 | 4/19/2023 | + (CDx) | 6/9/2023 | 12/5/23 | AFP: <3.0 | 6/20/23 | + | Both | | | |
| **3** | HCC | 8/10/2010 | 8/18/2010 | AFP: 8.7 | 9/24/2010 | AFP: 4.6 | | | 10/12/2010 | 10/21/2010 | AFP:7.1 | 12/19/2019 | + | Post- | 12/16/2019 | 12/17/2019 | AFP: 4398.6 |
| **4** | HCC | 12/18/2020 | 12/18/2020 | AFP: 6.2 | 3/7/2022 | AFP: 9.3 | | | 4/9/2022 | 6/21/2022 | AFP: <3.0 | 4/11/2023 | + | Post- | | | |
| **5** | HCC | 7/11/2022 | 1/27/2022 | AFP: 11 | 1/27/2022 | AFP: 11 | | | 7/11/2022 | 7/28/2022 | AFP: <3.0 | 8/10/2022 | + | Post- | | | |
| **6** | HCC | 2/18/2019 | 2/18/2019 | AFP: 7.6 | 2/3/2020 | AFP: 4.9 | | | 5/1/2020 | 10/23/2020 | AFP: <3 | 11/12/2021 | + | Post- | 10/23/2020 | 6/2/21 | AFP: <3.0 |
| **7** | HCC | 3/15/2022 | 3/15/2022 | AFP: 7.1 | 09/08/2022 | AFP: 9.5 | | | 9/18/2022 | 10/10/2022 | AFP: 10.5 | 11/14/2022 | + | Post- | 10/4/2023 | 10/4/23 | AFP: <3.0 |
| **8** | HCC | 11/8/2019 | 8/2/2019 | AFP: 5.6 | 2/20/2020 | AFP: 6.3 | 12/18/2019 | - | 4/12/2020 | 12/16/2020 | AFP: <3 | | | Pre- | | | |
| **9** | HCC | 7/19/2022 | 7/19/2022 | AFP: 14 | 12/8/2022 | AFP: 8.1 | 8/15/2022 | + | 12/30/2022 | 11/18/2022 | AFP: 6 | 9/1/2023 | - | Both | | | |
| **10** | HCC/CCA | 5/4/2021 | 5/4/2021; 5/20/2021 | AFP: 38.8, CA19-9: 216 | 7/5/2022 | AFP: 36.6, CA 19-9: 834 | 6/6/2022 | + | 7/7/2022 | 7/22/2022 | AFP: 4.8 | | | Pre- | | | |
| **11** | CCA | 7/14/2021 | 6/9/2021 | CA19-9: 8 | 1/12/2023 | CA 19-9: 46 | 7/20/2022, 9/2/22 | +, + | 2/7/2023, 07/13/23 | | | 9/28/2023 | - | Both | | | |
| **12** | CCA | 7/3/2020 | 6/19/2020 | CA19-9: 22 | 1/10/2023 | CA 19-9: 15 | | | 3/1/2023 | 7/31/2023 | CA19-9: 6.3 | 6/14/2022 | + | Post- | 11/3/2021 | 10/25/21; 5/25/21 | CA 19-9: 146; AFP: <3 |
| **13** | CCA | 11/25/2022 | 1/21/2021 | CA19-9: 24 | 8/4/2020 | CA 19-9: 45 | 12/13/2022 | - | 8/6/2020 | 12/1/2020 | CA19-9: 20 | 8/1/2023 | - | Both | | | |
| **14** | CRLM | 6/2017 | 12/10/2018 | CEA: 2.4 | 9/21/22 | CEA: 1 | 10/27/22, 9/25/23 | -, - | 10/11/23 | 12/11/23 | CEA: 1.6 | 11/1/23 | + | Both | | | |
| **15** | CRLM | 2/20/2020 | 3/3/2020 | CEA: 6854 | 8/9/2022 | CEA: 4.9 | 5/19/2022 | + | 9/14/2022 | 1/10/2023 | CEA: 1.8 | 11/15/2022 | - (GR) | Both | | | |
| **16** | CRLM | 10/5/2017 | 9/15/2017 | CEA: 60.1 | 1/6/2020 | CEA: 10.4 | 11/11/2019 | + | 1/12/2020 | 2/6/2020 | CEA: 1.2 | 1/12/2022, 7/15/22, 1/16/23 | -, -, - (GR) | Both | | | |
| **17** | CRLM | 2019 | 8/26/2021 | CEA: 1 | 10/28/2022 | CEA: 2.7 | 11/1/2022 | + | 11/1/2022 | 12/15/2022 | CEA: 0.9 | 12/8/2022, 6/7/23 | +, + | Both | | | |
| **18** | CRLM | 11/12/2011 | 8/23/2011 | CEA: 1.6 | 9/10/2020 | CEA: 1.6 | 6/25/2019 | + | 9/13/2020 | 1/9/2023 | CEA: 1.7 | 11/8/2021, 5/5/22 | - (GR) | Both | | | |
| **19** | CRLM | 4/1/2016 | 4/14/2016 | CEA: 64.4 | 11/27/2017 | CEA: 3.7 | | | 4/22/2018 | 5/24/2018 | CEA: 3.8 | 1/23/2020, 4/19/22 | +, + | Post- | | | |
| **20** | CRLM | 6/16/2012 | N/A | N/A | 5/27/2018 | CEA: 2.9 | | | 5/27/2018 | 8/30/2018 | CEA: 2 | 5/27/2022, 2/23/22, 7/28/23 | -, -, - (GR) | Post- | 9/19/2019 | 9/19/2019 | CEA: 1.8 |
| **21** | CRLM | 11/9/2020 | 11/2/2020 | CEA: 30.7 | 8/9/2022 | | 8/6/2022, 11/10/22 | +, - | 2/6/2023 | 7/3/23 | CEA: 16.5 | 10/31/23 | + | Both | 9/25/23 | 7/17/23; 9/25/23 | CEA: 17.2; CEA: 17.9 |

Key: CDx = Guardant CDx; GR = Guardant Reveal. All other ctDNA results are from Guardant360. AFP units = ng/mL, CA19-9 units = U/mL, CEA units = ng/mL. "+" and "-" symbols correspond to presence or absence of ctDNA respectively.

**Table 5.** Oncologic variables including treatment before and after liver transplant as well as with recurrence.

| Pt | Cancer Type | Liver Cancer dx | Pre-Transplant Treatment | Chemotherapy Details | Radiation Therapy Details | Surgery Details | Pathologic Response | Date of Transplant | Date of Recurrence | Recurrence, Number of Tumors, Sites | Largest Tumor Size (cm) | Treatment of Recurrence | Recurrence Treatment Details | Death, Cause |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **1** | HCC | 07/2022 | TARE | | 9/2022 | | PR | 8/15/23 | | | | | | |
| **2** | HCC | 2/16/2023 | - | | | | | 6/9/2023 | | No | | | | |
| **3** | HCC | 8/10/2010 | Microwave ablation | | | | | 10/12/2010 | 12/16/2019 | Intrahepatic; multifocal | 11.5 | Chemotherapy | 02/2/2020–11/6/2020: levatinib; switched to cobozanrinib after progression until 11/6/2020 | 12/17/2020; HCC |
| **4** | HCC | 12/18/2020 | TACE, TARE | 03/2/12, 5/11/21, 8/18/21 | 1/14/22 | | PR | 4/9/2022 | | No | | | | |
| **5** | HCC | 7/11/2022 | - | | | | | 7/11/2022 | | No | | | | |
| **6** | HCC | 2/18/2019 | TARE | | 09/19/2019, 11/19/2019 | | SD | 5/1/2020 | 10/23/2020 | Extrahepatic; multifocal—lung, adrenal fossa, retrocaval lymph nodes | 1.3 | Chemotherapy, radiation | 9/7/21: radiation; 12/3/21–2/3/22: levatinib | 5/13/2022: HCC |
| **7** | HCC | 3/15/2022 | TARE | | 5/18/22 | | SD | 9/18/2022 | 10/4/2023 | Extrahepatic; multifocal—lung | 1.5 | Chemotherapy | 11/22/23: levatinib | |
| **8** | HCC | 11/8/2019 | TACE | 12/2/2019 | | | PR | 4/12/2020 | | No | | | | 10/8/2023: metastatic melanoma |
| **9** | HCC | 7/19/2022 | SBRT | 09/26/22–10/10/22: 4 treatments | | | PR | 12/30/2022 | | No | | | | |
| **10** | HCC/CCA | 5/4/2021 | - | | | | | 7/7/2022 | | No | | | | |
| **11** | CCA | 7/14/2021 | Chemoradiation | 08/29/22–09/16/22: capecitabine | 08/29/22–09/16/22 | | SD | 2/7/2023, 07/13/23 | | No | | | | |

**Table 5.** *Cont.*

| Pt | Cancer Type | Liver Cancer dx | Pre-Transplant Treatment | Chemotherapy Details | Radiation Therapy Details | Surgery Details | Pathologic Response | Date of Transplant | Date of Recurrence | Recurrence, Number of Tumors, Sites | Largest Tumor Size (cm) | Treatment of Recurrence | Recurrence Treatment Details | Death, Cause |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **12** | CCA | 7/3/2020 | SBRT | 09/26/2019–09/27/2019 | | | CR | 8/6/2020 | 11/3/2021 | Extrahepatic; multifocal—liver, bone | | Chemotherapy, radiation | 9/6/22–9/21/22: radiation; 12/1/21–7/1/22: gemcitabine/oxaliplatin; 7/26/22–8/1/22: FOLFIRI; 10/1/22–12/1/22: gemcitabine/abraxane x 3 with PR | 3/15/23: cardiovascular event during dialysis; CCA |
| **13** | CCA | 11/25/2022 | Chemoradiation, SBRT | 1/10/23–2/3/23: capecitabine | 1/10/23–2/3/23 | | CR | 3/1/2023, adjuvant capecitabine x 4 cycles (6/5/23) | | No | | | | |
| **14** | CRLM | 2015 | Chemotherapy, surgery, microwave ablation | 7/11/17–9/20/17, 8/2019–5/8/2018: FOLFOX/cetuximab; 8/19–2/20: capecitabine, 8 cycles; 4/19/21–9/21: capecitabine | 5/18/22: microwave ablation; 2/2/23: SBRT 30 Gy in 1 fraction | 12/12/2017: open wedge resection (segments 4–8); 1/18/19: segment 4b lesion resection; 7/2/19: segment 8 lesion resection; 2/23/21: segments 7/8 liver resection | CR | 10/11/23 | | No | | | | |
| **15** | CRLM | 2/20/2020 | Chemotherapy, immunotherapy, radiation therapy | 3/20–8/18/20: CAPOX, bevacizumab; 10/2020–early 2021: 5FU, bevacizumab; 07–08/21: 5FU only; 10/21–01/22: 5FU, bevacizumab | 01–06/2021 | | CR | 9/14/2022 | | No | | | | |

**Table 5.** *Cont.*

| Pt | Cancer Type | Liver Cancer dx | Pre-Transplant Treatment | Chemotherapy Details | Radiation Therapy Details | Surgery Details | Pathologic Response | Date of Transplant | Date of Recurrence | Recurrence, Number of Tumors, Sites | Largest Tumor Size (cm) | Treatment of Recurrence | Recurrence Treatment Details | Death, Cause |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **16** | CRLM | 10/5/2017 | Chemotherapy, TARE | 10/2017–02/2018: FOLFOX, Avastin x 9 cycles; 02/18–12/11/19: FOLFIRI/panitumumab | 4 rounds | | PR | 1/12/2020 | | No | | | | |
| **17** | CRLM | 2019 | Chemotherapy, radiation therapy, SBRT | 09–11/11/2020: FOLFOX, Avastin x 12 cycles; 12/2020–05/2021: Avastin | SBRT: 9/20/2020 | | CR | 11/1/2022 | | No | | | | |
| **18** | CRLM | 11/12/2011 | Chemotherapy, radiation therapy, surgery, TACE, RFA | 10/18/2011–04/2012: Xeloda, FOLFIRI x 3 cycles; 07/2017: FOLFIRI, Erbitux; 02/25/15–03/2015: HAI pump infusion therapy | | Hepatic resection 02/25/2015 and 09/2016 | PR | 9/13/2020 | | No | | | | |
| **19** | CRLM | 4/1/2016 | Surgery, TACE, chemotherapy | 1/17/2015: HAI FUDR; 8/26/2016: FOLFIRI w/panitumumab x 6 cycles, FOLFOX Avastin x 3 cycles | | Wedge resection segments 2 and 3, caudate lobe removal, R hepatectomy | CR | 4/22/2018 | | No | | | | |

**Table 5.** *Cont.*

| Pt | Cancer Type | Liver Cancer dx | Pre-Transplant Treatment | Chemotherapy Details | Radiation Therapy Details | Surgery Details | Pathologic Response | Date of Transplant | Date of Recurrence | Recurrence, Number of Tumors, Sites | Largest Tumor Size (cm) | Treatment of Recurrence | Recurrence Treatment Details | Death, Cause |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **20** | CRLM | 6/16/2012 | Chemotherapy, ablation, TACE, radiotherapy | 08–10/2013: FOLFIRI; 12/2013: hepatic resection, HAI pump; until 10/2014: FUDR; 01–04/2014: 5FU; 05–01/2016: irinotecan, cetuximab; 02/2016–11/2017: 5FU cetuximab, 3/7/2018: FOLFOX x 13 cycles | 12/2017: proton beam radiother-apy | 07/2013: Ablation | * | 5/27/2018 | 9/19/2019 | Extrahepatic; unifocal, right upper lobe of lung | 0.9 | Chemotherapy, surgery | Right upper lobe metastectomy; 12/16/2019–7/27/2020: FOLFIRI, bevacizumab with complete response | |
| **21** | CRLM | 11/9/2020 | Chemotherapy, TACE | 5/2021: FOLFOX x 7 cycles; 6/28/22–11/7/22: irinotecan; 9/28/22–1/4/23: panitumumab; 3/2/22: infusional 5FU | | | PR | 2/6/23 | 9/25/23 | Intrahepatic and extrahepatic—lung nodule | | Chemotherapy, plan for surgery | 10/17/23: irinotecan, panitumumab | |

Key: SBRT = stereotactic radiation body therapy, TACE = trans-arterial chemoembolization, TARE = trans-arterial radioembolization, RFA = radiofrequency ablation, PR = partial response, CR = complete response, SD = stable disease. * = Information at OSH.

**Table 6.** Pre- vs. post-transplant mutational profiles of patients who underwent sequential ctDNA testing by cancer type.

| Patient # | Cancer Type | Time From Pre-op Testing to Surgery (Days) | Pre-op Somatic Alterations Detected | Pre-Transplant ctDNA | Time from Surgery to Post-op Testing (Days) | Post-op Somatic Alterations Detected | Post-Transplant ctDNA |
|---|---|---|---|---|---|---|---|
| 2 | HCC | 51 | Yes | CTNNB1 L31V 0.20% | 11 | Yes | CTNNB1 D32V N/A |
| 9 | HCC | 137 | Yes | *TERT* Promoter SNV 0.80%<br>*FGFR2* K509E 2.00% | 245 | No | Not Identified |
| 11 | HCC | 148 | Yes | Not Identified | 233 | No | Not Identified |
| 13 | CCA | 78 | No | Not Identified | 153 | No | Not Identified |
| 14 | CRLM | 16 | No | Not Identified | 21 | Yes | *ROS1* L1899F 0.2% |
| 15 | CRLM | 26 | Yes | *MTOR* Q1715 0.40%<br>*APC* E1064 * 0.50% | 62 | No | Not Identified |
| 16 | CRLM | 62 | Yes | *TP53* R248Q 0.10%<br>*SMAD4* A418fs 0.06%<br>*MAP2K1* K84R 0.20% | 731 | No | Not Identified |
| 17 | CRLM | 0 | Yes | *NF1* A706V 0.10%<br>*MLH1* I191 0.20% | 37 | Yes | *FGFR3* T317A 1.80%<br>*PALB2* N241D 1.60%<br>*BRCA2* C1290Y 1.50%<br>*ROS1* T632N 1.20%<br>*MET* V378I 0.10% |
| 18 | CRLM | 293 | Yes | *ROS1* A2106T 0.20%<br>*BRCA1* K22E 0.10% | 421 | No | Not Identified |
| 21 | CRLM | 184 | Yes | *APC* S1415fs 1%<br>*TP53* S149fs 1.3% | 266 | Yes | *APC* S1415fs 0.2%<br>*TP53* S149fs 0.2% |

Note: Percentages shown represent %cfDNA (cell-free DNA). N/A = not available. Asterisk (*) indicates unknown substitution.

**Table 7.** Tumor details from diagnostic radiologic imaging and explant pathology.

| Pt | Cancer Type | DxNumber of Tumors | Dx-Largest Tumor Size (cm) | Pathologic Tumor Numbers | Pathologic Largest Tumor Size (Viable) (cm) | % Viable Tumor Explanted Liver | Pathologic Vascular Invasion | Pathologic Perineural Invasion | Pathologic Liver Capsule Involvement | Histologic Grade of Differentiation | MSI | Pathologic TNM Staging from Transplant |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **1** | HCC | 1 | 3.6 | 3 | 0.8 | 20% | Small vessel | Absent | Absent | G2 | | T2 |
| **2** | HCC | 1 | 6.6 | 1 | 2.5 | 100% | Absent | Absent | Absent | G2 | | T1b |

**Table 7.** *Cont.*

| Pt | Cancer Type | DxNumber of Tumors | Dx-Largest Tumor Size (cm) | Pathologic Tumor Numbers | Pathologic Largest Tumor Size (Viable) (cm) | % Viable Tumor Explanted Liver | Pathologic Vascular Invasion | Pathologic Perineural Invasion | Pathologic Liver Capsule Involvement | Histologic Grade of Dif-ferentiation | MSI | Pathologic TNM Staging from Transplant |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **3** | HCC | 1 | 4 | 1 | 3 | 0% | Absent | Absent | Absent | G2 | | T1bN0 |
| **4** | HCC | 3 | 2.8 | 1 | 2.3 | 100% | Absent | Absent | Absent | G2 | | T2 |
| **5** | HCC | 5 | 1.7 | 5 | 1.7 | 100% | Small vessel | Absent | Absent | G2–3 | | T2 |
| **6** | HCC | 1 | 4.9 | 4 | 2.7 | 5% | Small vessel | Absent | Absent | G2 | | T2N0 |
| **7** | HCC | 1 | 4.2 | Multiple | 4.3 | 50% | Small and large vessel | Absent | Abuts | G2 | | T4 |
| **8** | HCC | 1 | 2.6 | 1 | 0.8 | 50% | Absent | Absent | Absent | G2 | | T1a |
| **9** | HCC | 1 | 8 | 1 | 2.3 | 20% | Absent | Absent | Absent | G2 | | T1b |
| **10** | HCC/CCA | 3 | 2.3 | 3 (2-HCC, 1-CCA) | 2-HCC, 10-CCA | 0%, 5%, 95% | Present | Present | Posterior capsule | G2–3 | | T2 |
| **11** | CCA | 1 | 1 | 1 | 0.1 | 100% | Absent | Absent | Absent | G1 | | T2aN0 |
| **12** | CCA | 1 | 1 | 0 | 0 | N/A | N/A | N/A | N/A | N/A | | |
| **13** | CCA | 1 | 1 | 1 (residual) | No gross lesion visible | | | | | G2 | | T1N0 |
| **14** | CRLM | Numerous | 7.6 | 0 | 0 | N/A | N/A | N/A | N/A | N/A | Stable | T0N1aM1 |
| **15** | CRLM | Numerous | 7.7 | 21 | 4.1 | 20% | Absent | Absent | Absent | | Stable | T3N1M1a |
| **16** | CRLM | 3 | 5.8 | 3 | 4 | 100%, 0% | Absent | Absent | Absent | | Stable | T3N1aM1 |
| **17** | CRLM | * | * | 1 | 8.5 | 0% | Absent | Absent | Absent | | Stable | T3N1aM1 |
| **18** | CRLM | 3 | * | 1 | 4 | 0% | Absent | Absent | Absent | | Stable | |
| **19** | CRLM | 2 | * | 0 | | | | | | | Stable | T3N1aM1 |
| **20** | CRLM | * | * | 4 | 1.7 | 100% | Absent | Absent | Absent | G2 | Unknown | |
| **21** | CRLM | 2 | 1.4 | 6 | 3.3 | 100% | Absent | Absent | Absent | G2 | Stable | T3N0M1 |

Key: * = imaging performed at OSH, Dx = diagnostic, N/A = not applicable.

## 4. Discussion

Liver transplant as a treatment for primary and secondary liver malignancy has grown in volume, with expansion from HCC to CCA and, more recently, to CRLM [6]. However, recurrence after LT remains a concern [22]. CtDNA has emerged as a non-invasive surveillance tool in predicting and detecting recurrence after the treatment of hepatic malignancies [23]. Compared to traditionally used tumor markers (e.g., CA19-9) which are notorious for their limited sensitivity and specificity, ctDNA offers a more individualized testing modality that can be used to predict recurrence-free survival at earlier time points, leading to guided decision-making for treatment selection [24,25].

This study demonstrates proof-of-concept for ctDNA testing in patients undergoing LT for primary and secondary liver cancers. We found a higher absolute recurrence rate in patients with positive post-transplant ctDNA. In patients who experienced recurrence, ctDNA was detected in all patients with active disease. Conversely, ctDNA was not detected in the one patient who achieved remission after recurrence. When comparing pre- vs. post-transplant ctDNA, clearance of ctDNA was observed in half of the patients who underwent sequential testing. An overall reduction in the TMB was also noted after LT. Interestingly, 30% of patients with sequential testing acquired new genomic alterations in post-transplant ctDNA, which may induce caution toward recurrent malignancy and/or the introduction of confounding genomic material that influences the interpretation of the results.

Our group previously published on the use of ctDNA in the context of hepatic resection for CRLM, showing how the detection of post-operative ctDNA was associated with an increased likelihood of disease recurrence [21]. Similarly, Tie et al. (2023) [24], Liu et al. (2023) [26], and Nishioka et al. (2022) [27] showed that post-operative ctDNA positivity predicts a reduced recurrence-free (RFS) and overall survival (OS) in patients undergoing hepatectomy for CRLM. The results of the GALAXY study further demonstrate the association of post-operative ctDNA with an increased recurrence risk and the ability to identify patients who derived benefits from adjuvant chemotherapy in patients with stage II or III CRC [28]. In patients with resected CCA, the preliminary results from Yoo at al. (2023) similarly show positive ctDNA status is predictive of a poor RFS [29]. In HCC, Wang et al. (2020) showed a reduced RFS with post-operative ctDNA assessed according to a panel of four hotspot genomic mutations in *TP53* (G747T), *CTNNB1* (A121G, C133T), and *TERT* (c.-124C>T) [30]. In the setting of liver transplant for unresectable primary liver cancer, larger scale studies by Huang et al. (2023) [23] and Jiang et al. (2022) [31] again display higher recurrence rates in patients with positive post-transplant ctDNA and decreased disease-free survival.

The widely known limitations of tumor serum biomarkers are additionally observed in our study. Of the six patients in our study who experienced recurrence, three (#6, 7, 20) had normal serum levels of traditionally used biomarkers at time of recurrence. However, ctDNA was detected post-transplant in two of these patients (#6, 7), demonstrating a potential set of patients in whom the recurrence of HCC following LT may be predicted or detected with ctDNA. To this end, expanding the enrollment of patients undergoing post-transplant ctDNA testing and conducting serial testing at earlier time points following LT may help elucidate whether the detection of ctDNA correlates with or predicts recurrence. If shown to be of prognostic utility, ctDNA could be used to stratify patients based on their risk of recurrence and determine more targeted, individualized selection of adjuvant therapy.

In addition, we report the acquisition of new mutations post-transplant in several patients who underwent sequential tumor-agnostic ctDNA testing. Although the exact source of the ctDNA is unknown, the absence of viable tumors in the explant histopathology for at least two patients may lead us to postulate that these mutations may be of donor origin. Alternatively, they may represent somatic mutations in the setting of immunosuppression post-transplant or clonal evolution. To address this concern, tumor-informed genetic testing may be considered due to its ability to differentiate ctDNA from germline-derived variants, clonal hematopoiesis of indeterminate potential, and dd-cfDNA. Such tumor-informed

tools have been developed and are actively being explored in clinical studies and trials [32]. However, these methods do have limitations in patients who have received extensive pre-LT locoregional and systemic therapy, as adequate viable tumor is necessary for tissue-informed testing. Given the uncertain origin of the novel post-LT genomic alterations, making ctDNA-based treatment decisions may be challenging in this subset. At a minimum, pre- and post-LT testing should be pursued when using tissue-agnostic testing in order to obtain a pre-transplant comparison. With expanding evidence supporting the use of ctDNA testing in liver cancers [24–31], the optimization of protocols effective at addressing the concerns regarding donor-derived alterations is warranted in future studies.

In addition to assessing for the presence of ctDNA, liquid biopsy can identify specific genes that predict patient outcomes based on cancer. For example, in HCC, *CTNNB1* and *TERT* have been shown to be two of the most commonly mutated genes and were present frequently in our cohort [33]. The presence of these two mutations, along with a mutation in *TP53*, in post-operative ctDNA has been associated with a decreased recurrence-free survival [30]. In CCA, the mutations are thought to be more heterogeneous, though mutations in *KRAS*, *IDH1/2*, *FGFR*, *ERBB2*, and *BRAF* have been noted to be more frequently mutated [34]. In colorectal cancer, mutations in *APC* and *TP53* are known to drive the transition from adenoma to adenocarcinoma [35–38]. In patient #21, the presence of these mutations post-transplant, although at lower variant allele frequencies, was detected prior to diagnosis of recurrence (Table 8). While our study was not aimed at addressing the prognostic or therapeutic implications of specific genes, the correlation between our findings in solid organ transplant patients and the published findings in the non-transplant population is encouraging for the application of liquid biopsy to this new set of patients. Tissue-agnostic ctDNA testing could theoretically provide such analysis before transplant, allowing for pre-transplant prognostication. One example of potential utility is the detection of mutations that are contraindications to transplant, such as *BRAF V600E*, which represents a contraindication to LT for CRLM in our center. As detection of such a mutation pre-LT may preclude transplant due to high risk of recurrence, the use of ctDNA in the transplant population warrants further investigation for optimization of protocols and interpretation.

**Table 8.** ctDNA profiles for patients who experienced recurrence.

| Patient Number | Cancer Type | Date Pre-Transplant ctDNA Collected | Pre-op Somatic Alterations Detected | Pre-Transplant ctDNA | Date Post-Transplant ctDNA Collected | Post-op Somatic Alterations Detected | Post-Transplant ctDNA | Date of Recurrence |
|---|---|---|---|---|---|---|---|---|
| 3 | HCC | | | | 12/19/2019 | Yes | *CTNNB1* T41A 3.70%<br>*TERT* Promoter 2.00% | 12/16/2019 |
| 6 | HCC | | | | 11/12/21 | Yes | *ARID1A* S696fs 0.70%<br>*CTNNB1* S33A 16.50%<br>*TERT* promoter 13.30% | 10/23/2020 |
| 7 | HCC | | | | 11/14/2022 | Yes | *TP53* R248Q 0.10%<br>*FGFR1* V247V 6.00% | 10/4/2023 |
| 12 | CCA | 12/3/22 | No | Not identified | 8/1/23 | No | Not identified | 11/3/2021 |
| 20 | CRLM | | | | 7/28/23 | No | Not Identified | 9/19/2019 |
| 21 | CRLM | 8/6/22 | Yes | *TP53* S149fs 1.30%<br>*APC* S1415fs 1.00%<br>*AR* R780W 0.50% | 10/31/23 | Yes | *APC* S1415fs 0.2%<br>*TP53* S149fs 0.2% | 9/25/23 |

Note: Percentages shown represent %cfDNA (cell-free DNA).

The limitations of this study include a small sample size, which is insufficient for determining causal relationships between ctDNA clearance and liver transplant. Furthermore, a low number of patients had sequential testing, which interferes with the evaluation of donor-derived cell-free DNA. Inconsistency in the ctDNA sampling and timing may have arisen due to challenges in clinical practice and logistics. To address these issues, a large-scale multi-institutional study is being conducted to increase the patient volume, and new institutional protocols have been implemented to ensure adequate sampling. Furthermore, the impact of neoadjuvant and adjuvant chemo, immune, and radiation therapy on the ctDNA results is still unknown. Lastly, the correlation of ctDNA with

tissue-based mutational profiles was not assessed in the present study, although concurrent tissue testing is now ongoing.

## 5. Conclusions

Circulating tumor DNA can help us to identify recurrence after liver transplant for hepatic malignancy. Transplantation was also associated with clearance of the ctDNA burden in half of the patients with sequential testing. We report a subset of patients with non-viable tumors and novel post-transplant genomic profiles, raising concern about donor-derived sources; improved approaches are necessary to address the potential of such findings confounding treatment decisions. Larger-scale studies and serial monitoring should be conducted to confirm the utility of ctDNA as a surveillance tool for MRD post-transplant and optimize the timing of the screening protocols.

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