Successful liver transplantation with ctDNA clearance after PD-1 inhibitor plus FOLFOX-HAIC treatment in HCC: A case report

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Abstract. Liver transplantation (LT) is the primary treatment for patients with early-stage hepatocellular carcinoma (HCC). However, the 5-year survival rate after LT remains low for patients with advanced HCC. Recently, combining programmed cell death protein-1 (PD-1) inhibitors with hepatic arterial infusion chemotherapy (HAIC) has achieved promising outcomes in advanced HCC treatment. However, there is a lack of sufficient clinical data demonstrating its effectiveness as a pre-LT down-staging treatment. The current study presented a case of advanced HCC beyond the Milan criteria who underwent LT and achieved a favorable outcome following PD-1 inhibitor combined with FOLFOX-HAIC therapy. Of note, due to treatment-induced tumor necrosis, precise post-treatment tumor size evaluation became challenging. To address this, circulating tumor DNA (ct-DNA) clearance was used as the LT criterion. After three cycles of Pembrolizumab and FOLFOX-HAIC therapy, the patient's serum ctDNA became undetectable and serum α-fetoprotein levels returned to normal. Magnetic resonance imaging results also revealed a significant reduction in liver tumor size post down-staging treatment. Subsequent to LT, serum ctDNA was monitored every two months, consistently yielding diminished results. There were no clinical signs of recurrence 19 months post-LT. These findings suggest that Pembrolizumab in combination with FOLFOX-HAIC may serve as a potential down-staging strategy prior to LT. In addition, ctDNA clearance may be considered a viable biomarker for LT eligibility.

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Introduction

Hepatocellular carcinoma (HCC) is an aggressive malignancy frequently diagnosed in advanced stages, with a median survival ranging from 6 to 20 months following diagnosis. In many countries, liver transplantation (LT) is the established therapy for early-stage HCC (1). However, individuals with advanced HCC, characterized by tumor numbers and sizes exceeding the Milan criteria, experience poor 5-year survival rates following LT (2). To address this challenge, down-staging treatment prior to LT has been widely adopted (3,4). In recent years, programmed cell death protein-1 (PD-1) inhibitors have demonstrated success in the management of solid organ cancers (5). Furthermore, continuous hepatic artery infusion chemotherapy (HAIC) has been explored as a primary intervention for patients with extensive HCC (6). Consequently, the combination of PD-1 inhibitors and FOLFOX-HAIC has achieved markedly improved outcomes in the treatment of advanced HCC. Although the efficacy of PD-1 inhibitors alongside FOLFOX-HAIC for HCC therapy is well-established (7), its effectiveness as a down-staging treatment before LT has not been adequately examined. The current study presented the case of a patient with advanced HCC surpassing the Milan criteria who underwent LT and achieved a favorable outcome through treatment with PD-1 inhibitors and FOLFOX-HAIC. Of note, the induction of tumor necrosis by FOLFOX-HAIC made it challenging to precisely assess the post-treatment tumor size. To address this issue, circulating tumor DNA (ctDNA) clearance was employed as the benchmark for LT candidacy.

Materials and methods

Hematoxylin and eosin (H&E) staining. The liver tissues were collected and embedded in paraffin after being fixed in 4% paraformaldehyde and dehydrated employing an increasing gradient of ethanol. Subsequently, 4-mm-thick sections were prepared and stained with H&E according to standard procedures.

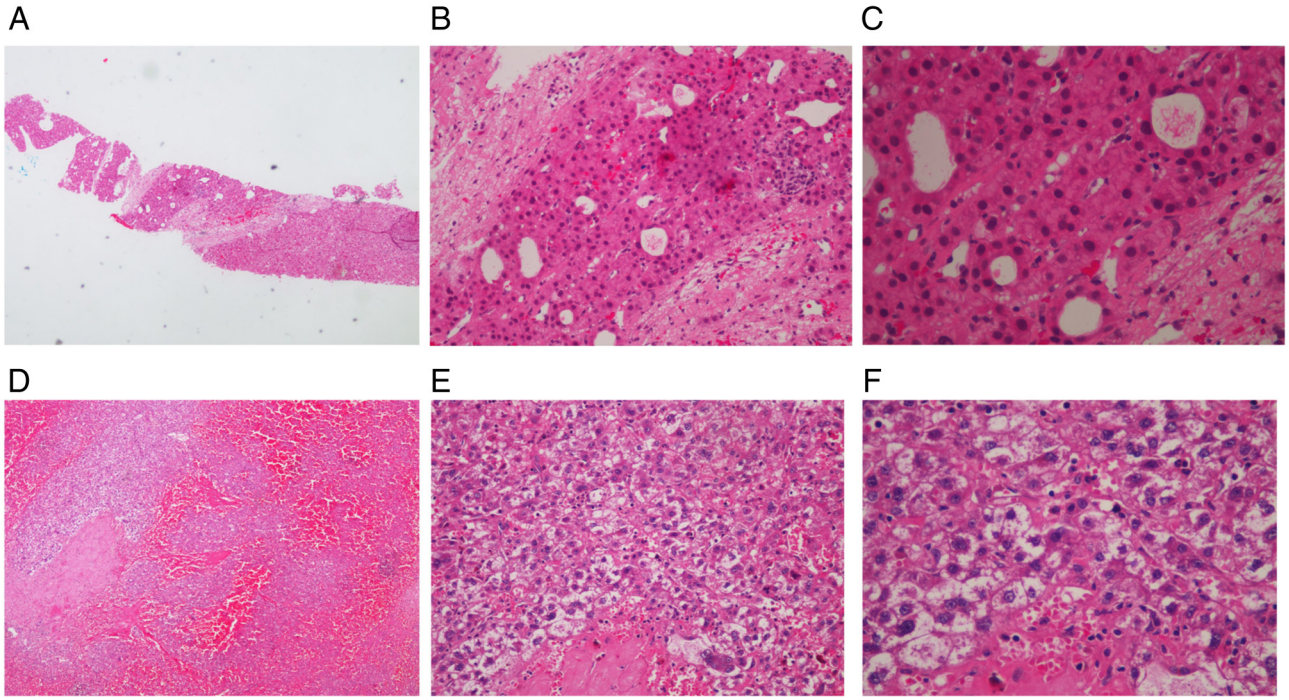

Figure 1. Pathological characteristics of the patient's liver: Liver biopsy and CT images before and after down-staging treatment prior to liver transplant. (A-C) Biopsy of the patient's liver before down-staging treatment displayed expanded hepatic sinusoids and broadened hepatic cords with adenoid changes [H&E; total magnification, (A) x4, (B) x20 and (C) x40]. (D-F) Biopsy of the patient's liver after down-staging treatment revealed a solitary tumor measuring 1.5 cm with some necrotic tissue [H&E; total magnification, (E) x4, (F) x20 and (G) x40].

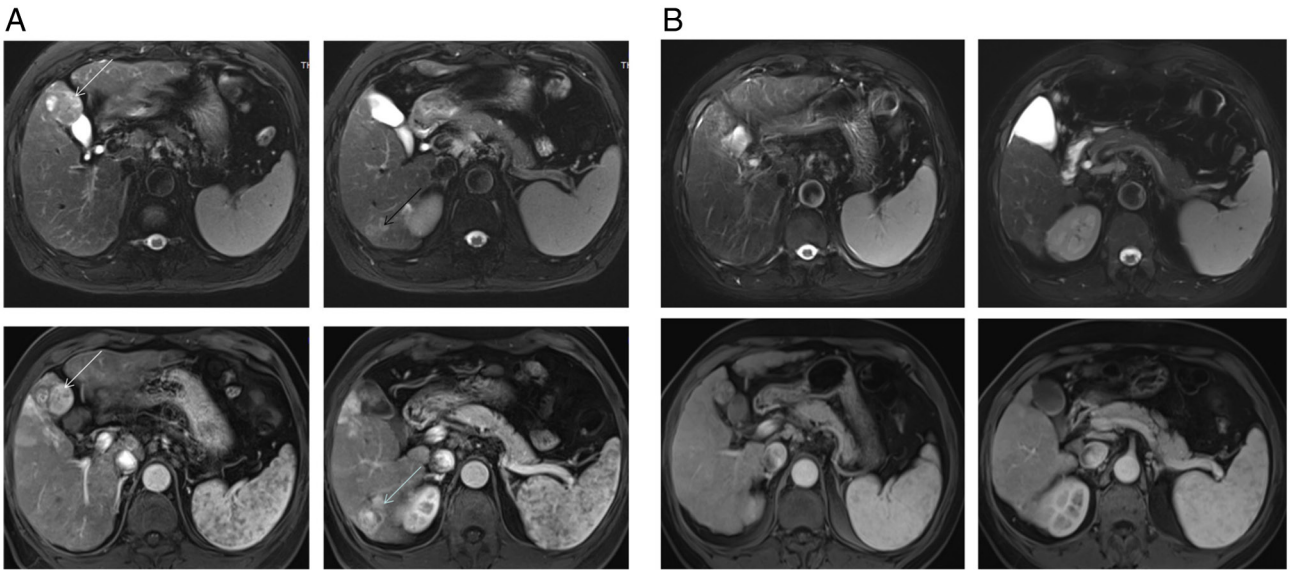

Figure 2. Imaging characteristics of the patient's liver. (A) MRI of the patient's liver before down-staging treatment. (B) MRI of the patient's liver after down-staging treatment. Arrows in the figure indicate the tumors.

Case report

A 55-year-old male patient, diagnosed with primary liver cancer, presented at Tongji Hospital (Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China) in July 2021 with a medical history of HBV infection and viral liver cirrhosis spanning over two years. The patient had been under antiviral treatment and regular examinations until July 2021. A liver biopsy confirmed the presence of HCC (Fig. 1A-C). The biopsy result after FOLFOX-HAIC treatment is presented in Fig. 1D-F. Enhanced CT scans revealed multiple liver tumors (at least 3 tumors were clearly visible), primarily on the right lobe, with the largest measuring ~3.3x2.6 cm, and enlarged lymph nodes in the post-peritoneum (Fig. 2A). In addition, the patient's α-fetoprotein (AFP) (41.97 ng/ml; normal level, ≤7.0 ng/ml according to the standard at our center) and transaminase showed slight elevation (ALT: 48 U/l; normal level, ≤41 U/l; AST: 55 U/l; normal level, ≤40 U/l, according to the

Table I. Characteristics of the patient's serum ctDNA before and after transplantation.

Time	Serum ctDNA
Before down-staging treatment	*TSC2* (S1723* c.5168C>A), *RB1* (c.1215+1G>A), *ATP2B3* (V415L c.1243G>C), *NPAP1* (H613L c.1838A>T), *DCTN1* (V844L c.2530G>T)
After down-staging treatment	Negative
7 days after LT	Negative
3 months after LT	Negative
12 months after LT	Negative

LT, liver transplant; ctDNA, circulating tumor DNA.

standard at our center). Due to the invasiveness of the method of liver biopsy, none was performed prior to neoadjuvant therapy. Given these findings, it was determined that the patient did not meet the Milan criteria (8) and down-staging treatment was initiated before LT using a combination of PD-1 inhibitors and FOLFOX-HAIC. Specifically, the patient received intravenous Pembrolizumab (Merck & Co., Inc.) every three weeks at a dosage of 2 mg/kg. Simultaneously, HAIC with the FOLFOX regimen was administered every three weeks as follows: Day 1-oxaliplatin (Sanofi®) 85 mg/m^2, leucovorin (Pfizer, Inc.) 400 mg/m^2 and 5-fluorouracil (Jinyao Pharmaceutical Co.) 400 mg/m^2 via intra-arterial infusion; days 2 and 3-5-fluorouracil 2,400 mg/m^2 via continuous intra-arterial infusion. The patient completed three treatment cycles with Pembrolizumab plus FOLFOX-HAIC. At the time of conclusion of these treatments, certain areas of the tumors had undergone necrosis and there was no sign of progression (Fig. 1D-F). Both AFP and transaminase (AST and ALT) levels had returned to normal. However, due to the tumor necrosis caused by FOLFOX-HAIC treatment, it was challenging to precisely assess the tumor size post-treatment. To address this issue, the patient's serum ctDNA (performed by the company YuBio®) was examined before and after down-staging treatment. Before the treatment, the patient's serum ctDNA result was positive (Table I). Following three cycles of Pembrolizumab plus FOLFOX-HAIC treatment, the patient's serum ctDNA became negative (Table I) and AFP levels returned to normal. However, it was difficult to measure the sum of the size of the enhanced lesion based on dynamic magnetic resonance imaging (MRI) images. In addition, MRI results indicated a significant reduction in liver tumor size after down-staging treatment (Fig. 2B). Based on these findings, it was determined that the patient showed partial response (9) and now met the Hangzhou criteria for LT (8), and the LT was performed in November 2021 (101 days after the commencement of down-staging treatment and 31 days after the last Pembrolizumab plus FOLFOX-HAIC treatment). The patient received a whole LT from a 61-year-old male deceased donor. The donor graft was procured through the Red Cross Society of Hubei Province and allocated via the China Organ Transplant Response System. The procedures were conducted in accordance with the national deceased organ donation program in China (10). It was assumed that the enlarged retroperitoneal lymph node was lymph node reactive hyperplasia. Therefore, it was not removed during LT. A biopsy performed immediately post-LT identified only one tumor with necrotic tissue (1.5 cm) on the patient's old liver. The patient received Basiliximab (20 mg; Novartis International AG) on post-operative days 0 and 4. After LT, the patient was administered immunosuppressive treatment consisting of tacrolimus (with the blood concentration maintained at 1-2 ng/dl; Astellas Pharma, Inc.) in combination with sirolimus (1 mg/day; Pfizer, Inc.) for maintenance. Serum ctDNA testing was conducted after three months and all results were negative (Table I). The patient did not experience any rejection up to the latest follow-up in April 2023 and maintained normal liver function and AFP levels (Fig. 3). The ctDNA results were in agreement with the other examination results, such as serum AFP levels. There were no clinical indications of recurrence at the 19-month mark post-LT. The patient remains in good health with normal liver function to date. Routine follow-up has been on-going at our center. It is important to note that the present study only reported the therapeutic outcome for a single patient. Further research is required to validate the treatment's effectiveness in larger studies.

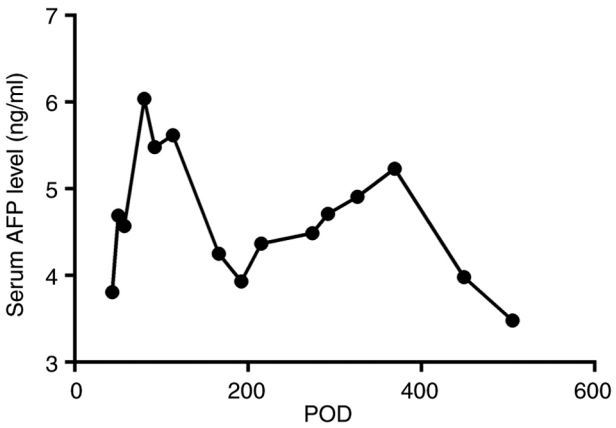

Figure 3. Serum AFP levels after liver transplantation. AFP, α-fetoprotein; POD, postoperative days.

Discussion

Although the overall survival of patients with HCC has improved with advancements in diagnosis and treatment, the majority of Asian patients are diagnosed at an advanced stage, necessitating more aggressive approaches (11). At our center, nearly half of the

patients on the LT waiting list have HCC and >70% of patients exceed the Milan criteria. In China, the Hangzhou criteria (maximal tumor diameter ≤8 cm, regardless of AFP levels and histopathological tumor differentiation, or a total tumor diameter >8 cm with AFP levels ≤100 ng/ml and well to moderately differentiated histopathological tumor grading G1 or G2) are widely accepted and utilized (8). Down-staging strategies are increasingly employed to identify patients suitable for LT based on their response to locoregional therapy (4). Previously, transhepatic artery chemoembolization (TACE) was commonly used for advanced HCC, offering hope to patients ineligible for radical surgery at initial diagnosis (12). It has been demonstrated that FOLFOX-HAIC significantly enhances overall survival compared to TACE in patients with large unresectable HCC (13). Immune checkpoint inhibitors have proven to be safe and effective in combating unresectable HCC (14). Consequently, PD-1 inhibitors combined with FOLFOX-HAIC have also been applied in the treatment of advanced HCC. In the present case, three cycles of Pembrolizumab plus FOLFOX-HAIC were administered, yielding favorable outcomes. As such, this combination may be proposed as a potential down-staging regimen before LT. However, the safety of immune checkpoint inhibitors for patients awaiting LT remains a subject of debate. For instance, Nordness *et al* (14) reported a case in which nivolumab was used pre-transplant for HCC, resulting in fatal acute hepatic necrosis. Furthermore, immunotherapy has been discussed as a neoadjuvant treatment before LT (15). However, severe complications such as acute allograft rejection may lead to graft loss and even mortality (16). The serum half-life of nivolumab in cancer patients is ~27 days, but clinical practice may exhibit variability among patients (17). Therefore, further research is warranted to confirm the safety of immune checkpoint inhibitors before LT.

Numerous studies have demonstrated that ctDNA analysis can detect post-treatment molecular residual disease in patients with localized lung cancer and identify residual/recurrent disease earlier than standard radiological imaging (18,19). However, there is a lack of data regarding its effectiveness in HCC. In the present case, the patient's ctDNA was analyzed before down-staging treatment, after down-staging treatment prior to LT and three months after LT. The current findings suggest that ctDNA clearance may serve as a potential biomarker or criterion for LT. In the future, larger-scale studies are necessary to further validate the utility of ctDNA as a biomarker for HCC detection.

In conclusion, the patient remains in good health with normal liver function to date. Routine follow-up was on-going in our center. Of course, the current case study only reported on the therapeutic effect in one patient. More research is needed to verify the therapeutic effect in further studies.

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Availability of data and materials

The data generated in the present study may be requested from the corresponding author

Authors' contributions

YYZ and DC analyzed and interpreted the data, and wrote the paper. LW and ZSC made substantial contributions to the conception and design of the study, revised the manuscript critically for important intellectual content, and gave the final approval of the version to be published. BY, JX, LW and GBH collected the data. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

The authors take full responsibility for all aspects of the research, including ensuring the investigation and resolution of any questions concerning the accuracy or integrity of any part of the work. This research, involving human participants, adhered to the Declaration of Helsinki (2013) guidelines.

Patient consent for publication

Informed consent was obtained from the patient.

Competing interests

The authors declare that they have no competing interests.

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