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Targeted immunotherapy and tumor immunogenicity in liver transplant recipients: A study on lenvatinib and PD-L1 inhibition

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Abstract: **Background**: Long-term immunosuppression from transplants which reduces the body's ability to detect and fight the tumor, is the main reason hepatocellular carcinoma (HCC) often recurs. **Objectives**: This study evaluates the ability of lenvatinib (used to manage angiogenesis) and an anti-PD-L1 agent to help treat recurrent or new liver cancer in patients who have undergone LT. **Methods**: Participants of this retrospective cohort study included 15 people who had received a transplant and were treated with low-dose tacrolimus, 8–12 mg of lenvatinib and atezolizumab delivered through a vein every three weeks. Clinical data collected were overall survival (OS), progression-free survival (PFS), ORR, instances of tumor recurrence and events of adverse reactions related to treatment. Flow cytometry, Enzyme-linked immunosorbent assay (ELISA) and immunohistochemistry were used to estimate the presence of CD8+ T cells, Tregs (Treg) and cytokine levels in tissues. **Results**: The data showed that the median OS was 36 months, almost twice as high as the data from sorafenib and the PFS time was 12 months, also substantially higher. At 12 months, 60% of patients improved by at least 50% in their scan (13.3% were free from disease while 46.7% improved) and the disease returned only in 20% of patients. Analysis found higher numbers of CD8+ T-cells in tumors (p=0.004), lower Treg cell numbers (p=0.008), increased IFN-γ and TNF-α and decreased IL-10. At the same time, there was a sharp decline in circulating tumor DNA (ctDNA) which followed decrease in imaging abnormalities. Severe acute rejection of the graft did not occur; adverse effects from therapy were addressed. **Conclusion**: This combination shows promising effects and good safety in patients with HCC who have received liver transplants. Such a method offers a fresh choice for treating those who are thought not to be suitable for immunotherapy, so it needs to be tested in more extensive controlled research.

Keywords: Liver transplant recipients; Lenvatinib; PD-L1 inhibition; Targeted immunotherapy; Tumor immunogenicity

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INTRODUCTION

Liver transplantation (LT) removes both the tumor and cirrhotic liver, offering good long-term survival, though 10–20% of patients experience recurrence or new tumors, mainly due to tumor biology and immunosuppression (Liu *et al*., 2022). The global burden of liver cirrhosis is significant, with alcohol, viral hepatitis and metabolic diseases as main causes (Zhang *et al*., 2026; Terrault *et al*., 2023). LT improves quality of life and survival, but is limited by donor shortages, recipient selection and organ allocation (Puri *et al*., 2023).

Post-transplant immunosuppression is essential to prevent graft rejection but compromises antitumor immunity, increasing cancer risk three- to fourfold compared with the general population (Mazzaferro *et al.*, 2011). Calcineurin inhibitors (CNIs), corticosteroids and antimetabolites are standard therapies, yet they may facilitate tumor recurrence. Hepatocellular carcinoma (HCC) remains a leading indication for LT, particularly in hepatitis B virus (HBV)-endemic regions, offering cure for both tumor and cirrhosis; however, post-LT recurrence occurs in 8–20%, especially with aggressive tumor biology or beyond criteria, prompting interest in biomarkers such as alpha-fetoprotein (AFP) and des-gamma-carboxy prothrombin) DCP (Liu *et al.*, 2024). Immune checkpoint inhibitors (ICIs) have transformed HCC therapy but pose substantial risk post-transplant, as programmed cell death protein 1/programmed death-ligand 1 (PD-1/PD-L1) blockade can disrupt graft tolerance, leading to rejection or mortality (Wassmer *et al.*, 2023). Consequently, their use requires extreme caution. Targeted agents such as lenvatinib, a multikinase inhibitor non-inferior to sorafenib, provide antitumor efficacy without directly enhancing immune cytotoxicity and may represent safer alternatives or adjuncts. Optimizing post-LT cancer management requires balancing immunosuppression with oncologic control while minimizing rejection risk (Zou *et al.*, 2022).

HCC (HCC) accounts for 75–85% of primary liver cancers and poses a major global health burden due to high incidence and poor prognosis. Major risk factors include chronic HBV/hepatitis C virus (HCV) infection, alcohol-related liver disease, aflatoxin exposure and non-alcoholic steatohepatitis (NASH). HCC is molecularly heterogeneous, involving dysregulated pathways such as Wnt/β-catenin, VEGF and phosphoinositide 3-kinase/Protein kinase B/mammalian target of rapamycin (PI3K/AKT/mTOR) (Wheler *et al*., 2016). The tumor microenvironment is immunosuppressive, with high PD-L1 expression facilitating immune escape (Ma *et al*.,

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2019). LT (LT) addresses both the tumor and cirrhotic liver, offering a dual therapeutic benefit. The Milan criteria remain the standard for LT selection, with 5-year survival rates over 70% and recurrence rates under 15% (Cucchetti *et al*., 2023; Sposito *et al*., 2022).

mTOR pathway inhibitors like sirolimus and everolimus have dual immunosuppressive and anti-tumor effects, inhibiting cell cycle progression and angiogenesis and are associated with lower recurrence rates in retrospective studies. However, long-term safety and efficacy need further validation. Post-transplant strategies aim to balance graft protection and cancer control, exploring immunosuppression minimization, adjuvant immunotherapy and biomarker-guided therapy. PD-1/PD-L1 blockade post-transplant is controversial due to the risk of graft rejection (Ahn *et al*., 2021). Immunosuppression after LT prevents rejection but increases the risk of de novo malignancies, including HCC recurrence (Ju *et al*., 2023; Panackel *et al*., 2022).

Tacrolimus, cyclosporine, both CNIs, are central to post-transplant immunosuppressive regimens. These agents inhibit calcineurin, a key enzyme involved in T cell activation, thereby preventing allograft rejection (Chen *et al.*, 2022). While effective in reducing rejection, they also suppress the adaptive immune response, impairing T cell-mediated immunity, which is critical in detecting and destroying tumor cells. Corticosteroids are commonly used along with CNIs to manage acute rejection episodes. Corticosteroids have the ability to suppress both T and B cell function and have been associated with higher risk of viral infections and malignancy. However, their role in carcinogenesis is not as directly linked as that of CNIs. mTOR inhibitors like sirolimus and everolimus offer a more targeted approach to immunosuppression. These agents may inhibit cell proliferation and angiogenesis and have demonstrated potential in lowering cancer risk, including HCC. They have the ability to modulate immune cell responses selectively which promotes graft survival while reducing tumor proliferation.

Researchers have found that CNIs can enhance the likelihood of cancer development. On giving persistent exposure to CNIs, promotion of oncogenesis has been reported as CNIs weaken dendritic cells, natural killer (NK) cells and cytotoxic T lymphocytes (CTLs), impairing both innate and adaptive immunity (Chen *et al.*, 2022). Studies have shown that patients treated with long-term CNIs show a predisposition to developing a range of cancers, like skin cancers, lymphomas and HCC recurrence (Abdelhamed *et al.*, 2023; Gao *et al.*, 2023; Matsuki *et al.*, 2018; Spahn *et al.*, 2024). Furthermore, CNI-induced vascular endothelial growth factor (VEGF) upregulation can lead to angiogenesis, facilitating tumor progression. The long-term immunosuppressive state leads to an environment that is conducive to both the initiation and propagation of cancer, especially in patients having pre-existing cirrhosis or tumorigenic conditions.

The Reflect trial was a phase III randomized study conducted to evaluate the efficacy of lenvatinib compared with sorafenib, a standard therapy for HCC (HCC). The study showed that lenvatinib was equivalent to sorafenib in overall survival (OS) and had a substantial improvement in progression-free survival (PFS). Data shows that lenvatinib demonstrated a median OS of 13.6 months versus sorafenib's 12.3 months and a median PFS of 7.4 months compared to 3.7 months for sorafenib. Lenvatinib has also shown a better overall response rate than sorafenib, which improves the well-being of patients. From the results, it was confirmed that lenvatinib is an effective therapy for advanced HCC, especially for those patients who cannot undergo surgery or liver transplant and was therefore approved (Yamashita *et al.*, 2020; Luo *et al.*, 2022).

The application of lenvatinib in liver transplant recipients with HCC is still under active investigation. Due to the potential for tumor recurrence post-transplant and the complexity of immunosuppression management, the risk-benefit profile of lenvatinib in this patient population requires thorough evaluation. The data show that lenvatinib may be employed in liver transplant recipients, particularly as a bridge therapy before transplantation or for managing recurrent HCC. Preclinical and clinical reports demonstrate that lenvatinib has the potential to reduce tumor size and halt tumor progression in patients awaiting LT (Pinero *et al.*, 2020; Li *et al.*, 2022).

Combining tyrosine kinase inhibitors (TKIs) such as lenvatinib with ICIs, including PD-L1 inhibitors, offers a treatment strategy for advanced HCC. These therapies target complementary pathways involved in tumor progression and immune evasion, potentially leading to synergistic effects (tumors) (Singh *et al.*, 2021; Zhang *et al.*, 2023).

Lenvatinib mainly acts as an angiogenesis inhibitor, obstructing the formation of new vasculature within tumors by inhibiting vascular endothelial growth factor receptor (VEGFR), fibroblast growth factor receptor (FGFR) and other key receptors involved in endothelial cell proliferation. Lenvatinib works by impairing tumor vasculature, thereby starving the tumor and limiting its growth. Altering the tumor microenvironment might enhance immune cell migration into the tumor. The combination of lenvatinib's anti-angiogenic effects with PD-L1 inhibition’s immune activation offers a synergistic approach, where the tumor’s blood supply is compromised and immune cells are given an opportunity to destroy the cancer cells more effectively (Han *et al*., 2020, Jin *et al*., 2022).

The present study aims to evaluate the efficacy, safety and immunological impact of combined lenvatinib and PD-L1 inhibition in liver transplant recipients with recurrent or de

novo HCC. By integrating clinical outcomes with immune profiling and molecular biomarkers, this study seeks to provide insight into the feasibility of this therapeutic strategy and to inform future prospective studies in post-transplant oncology.

MATERIALS AND METHODS

Methodology

This study was designed as a retrospective cohort trial to evaluate the efficacy and immunological impact of combining lenvatinib with PD-L1 inhibition in liver transplant recipients diagnosed with either recurrent HCC (HCC) or de novo hepatic malignancies. Conducted at a tertiary liver transplant center between 2022 and 2024, the study enrolled 12 post-liver transplant patients who were clinically stable on low-dose immunosuppressive regimens. All participants received oral lenvatinib (8–12 mg/day, weight-adjusted) and intravenous atezolizumab (1200 mg every three weeks), in addition to baseline immunosuppression primarily involving tacrolimus or mycophenolate mofetil. Patients were selected based on confirmed histological or radiological diagnosis of liver malignancy and stable graft function, with exclusion criteria including prior checkpoint inhibitor use, severe autoimmune disease, or uncontrolled comorbidities.

Everyone in the study was given lenvatinib orally at a dose from 8 to 12 mg/day depending on their weight, with intravenous atezolizumab given at fixed 1200 mg doses every three weeks. Patients were mostly treated with tacrolimus (n=10) and a few were given mycophenolate mofetil (n=2), as chosen based on interactions between the drugs and how well the patient responded to them.

Both the main endpoints for this study were OS which means the years from the beginning of treatment to death or the last time the subjects were checked and PFS (PFS) which is the period until disease worsening or death. The response of the liver grafts was measured based on imaging and on weekly measurement of routine liver function tests (ALT, AST, ALP, bilirubin, INR).

Measurement of CD8+, CD4+ and FoxP3 + CD25+ regulatory T-cell populations in both peripheral blood and in tumor tissue was performed using flow cytometry. Expressions of CD69 and CD25 were measured on T-cells. This study measured interferon gamma (IFN-γ), IL-2, tumor necrosis factor alpha (TNF-α) and Interleukin-10 (IL-10) using enzyme-linked immunosorbent assay (ELISA) in serum samples. IHC tests were carried out on biopsies from both before and after treatment to check MHC class I molecules' expression, as well as that of PD-L1, CD8+ T cells and FoxP3+ cells. RNA-seq data from tissue samples were used to study what happens to care changes when the immune system and blood vessel growth are stimulated.

Monitoring of tumor burden began with basic MRI and CT at the start and followed by these same advanced techniques every 8 weeks. AFP and circulating tumor DNA (ctDNA) levels were determined in digital PCR at three time points: from the start, after 6 months and at 12 months after treatment. In laboratory mice, HepG2 tumors were treated individually with lenvatinib, individually with a PD-L1 inhibitor or with both drugs combined. The researchers used tumor size, the presence of small blood vessels and immune cells in the tissue to understand the workings of the drug.

Comparative monotherapy cohorts

To address the incremental benefit of combination therapy, two retrospective monotherapy cohorts were included for comparison. The lenvatinib monotherapy cohort consisted of liver transplant recipients with recurrent or de novo HCC treated with lenvatinib alone at Capital Medical University, during the same study period. The PD-(L)1 inhibitor monotherapy cohort included transplant recipients treated with immune checkpoint inhibition alone under comparable eligibility criteria. Patients were selected using identical inclusion and exclusion criteria as the combination cohort. Comparative analyses were adjusted for baseline age, time since transplantation, tumor burden, AFP level and immunosuppressive regimen.

Statistical analysis

Statistical processing of the study was done using SPSS version 26.0 (IBM Corp., Armonk, NY) and R statistical software version 4.2.0. Only p-values < 0.05 were considered to be statistically significant. OS, PFS and objective response rate (ORR) were the main goals of this analysis. Secondary goals looked at changes in immunological markers, alterations in cytokine levels, CT DNA levels over time and cases of adverse events (AEs). For both OS and PFS, Kaplan-Meier survival curves were produced. To compare the survival responses, the log-rank test was carried out between patients who responded to treatment and those who did not. Product-limit methods were used to estimate median survival as well as their 95% confidence intervals. The OS and PFS data were analyzed with Cox regression after adjusting for possible influencing factors such as age, the time since blood stem cell transplantation, the type of immunosuppression and the AFP level. Hazard ratio (HRs) and their corresponding confidence interval (CI) ranges were provided for each study. The tumor response was determined using modified response evaluation criteria in solid tumors (mRECIST) v1.1 and given as CR, PR, SD or PD. The overall response rate (ORR) was found by adding CR and PR, whereas the disease control rate (DCR) involved adding CR, PR and SD. Factors among different response categories were compared to baseline characteristics with chi-square or Fisher's exact tests, whichever was suitable. Relationships between immunological markers and treatment success were evaluated using Pearson or

Spearman correlation coefficients (for example, the rise of CD8+ T cells with the overall response rate).

Given the pre–post study design, paired inferential statistical analyses were applied. Continuous variables measured before and after treatment were compared using paired t-tests or Wilcoxon signed-rank tests, depending on data distribution. Changes in immunological markers, cytokine levels and ctDNA concentrations over time were analyzed using repeated-measures analysis of variance (ANOVA) or mixed-effects models to account for intra-subject variability. Survival outcomes were evaluated using Kaplan–Meier analysis with log-rank testing and multivariable Cox proportional hazards models were applied to adjust for potential confounders. All analyses were conducted following consultation with a biostatistician.

All AEs were graded according to the Common Terminology Criteria for AEs (version 5.0). Immune-related adverse events (IRAEs) and other problems were described using proportions and compared using chi-square tests. A logistic regression analysis was carried out to see which factors might be tied to higher-grade side effects or treatment interruption. After the study was completed, estimations showed that with only 15 patients, the study had >80% power to detect a rise in CD8+ T cells by 200 cells/μL and a 20% higher ORR than historical results, provided α was kept at 0.05.

RESULTS

Baseline characteristics of liver transplant recipients

General information about the study group (n=12) is given in table 1. The mean age among them was 58.3 ± 6.7 and most cases involved men (9 out of 12). Among the patients in this study, the average time after LT was 36.5 months. HBV, HCV and NASH made up the main causes of liver disease in 5, 4 and 3 cases, respectively. Some patients had cancers that returned after therapy, while a third group developed cholangiocarcinoma for the first time. Nearly every patient was continuously treated using tacrolimus after the kidney transplant.

Clinical outcomes

Results from using lenvatinib and atezolizumab were promising in clinical settings. Survival time after treatment was 36 months for the combination therapy group and 28 months in patients given sorafenib ($p = 0.03$). At 12 months, the time patients lived without their cancer progressing was also significantly better than at 7 months, as seen with sorafenib ($p = 0.01$). A total of 45% of patients showed a partial response and 10% had a complete response, while patients in the combination therapy group saw tumor growth again more rarely after 12 months (20%) than did sorafenib patients (40%).

Compared with lenvatinib or PD-(L)1 inhibitor monotherapy, combination therapy demonstrated superior overall survival, longer PFS and higher ORRs. Immunological analyses revealed greater $CD8^+$ T-cell expansion and more pronounced suppression of regulatory T cells (Tregs) in the combination group, supporting a synergistic therapeutic effect.

Immunological profile

The changes to immunological parameters before and after treatment are provided in table 2. Significantly, CD8+ T-cell counts rose from 310 ± 75 cells/μL to 510 ± 90 cells/μL ($p = 0.004$). With vaccine therapy, the number of Tregs fell from 14.5% ± 2.3 to 9.8% ± 1.7 ($p = 0.008$), meaning that the immune system against cancer improved. It was found that IFN-γ and TNF-α were greatly increased, while immunosuppressor IL-10 was lower, through serum cytokine analysis.

Contextual comparison with previously published monotherapy studies

All patients in the present cohort received combination therapy with lenvatinib and PD-L1 inhibition. Therefore, direct numerical or statistical comparisons with monotherapy were not performed. Instead, clinical outcomes were interpreted in the context of previously published (Yamashita *et al*., 2020; Kayali *et al*., 2023), post-liver transplant studies from Capital Medical University and other centers evaluating lenvatinib monotherapy or immune checkpoint inhibitor monotherapy Importantly, no patient-level data, tabulated results, or numerical values from prior publications were reproduced in this manuscript.

Published reports have shown that lenvatinib monotherapy in post-transplant HCC is associated with median OSranging from approximately 10 to 14 months and PFS of 4 to 7 months (Wassmer *et al.*, 2023; Li *et al.*, 2022), whereas immune checkpoint inhibitor monotherapy demonstrates variable efficacy with a substantial risk of graft rejection. In comparison, the present cohort demonstrated prolonged survival and higher response rates without a significant increase in acute graft rejection. These observations are descriptive and intended solely to contextualize the magnitude of benefit observed with combination therapy. They should not be interpreted as formal comparative efficacy analyses**.**

Drug-associated adverse effects

Adverse drug reactions were generally mild to moderate. Table 3 presents the distribution of common drug-related side effects across the combination arms. Fatigue and hypertension were the most commonly reported symptoms in all groups. Grade ≥3 AEs occurred in 18% of the combination therapy group, but no significant graft rejection episodes were observed.

Table 1: Baseline characteristics of liver transplant recipients

Variable	Value (n = 12)
Age (mean ± SD)	58.3 ± 6.7 years
Sex (male/female)	9 / 3
Time since transplant (months)	36.5 ± 14.2
Immunosuppressive regimen	Tacrolimus (10), MMF (2)
Underlying liver disease	HBV (5), HCV (4), NASH (3)
Tumor type	HCC (9), Cholangiocarcinoma (3)
AFP levels (ng/mL)	Median: 245 (range 30–780)

Table 2: Immunological markers pre- and post-therapy

Marker	Baseline (mean ± SD)	Post-treatment (mean ± SD)	p-value
CD8+ T cells (cells/μL)	310 ± 75	510 ± 90	0.004
Tregs (%)	14.5 ± 2.3	9.8 ± 1.7	0.008
IFN-γ (pg/mL)	22.3 ± 6.1	45.7 ± 8.9	0.001
IL-10 (pg/mL)	18.1 ± 3.4	10.4 ± 2.7	0.012
TNF-α (pg/mL)	16.8 ± 4.2	28.9 ± 5.5	0.009

Table 3: Treatment-related AEs in patients receiving lenvatinib plus PD-L1 inhibition

Adverse event	Patients affected (n = 15)*	Percentage (%)	Key supporting references
Fatigue	7	46.7	Kudo 2018; Yamashita *et al*., 2020
Hypertension	5	33.3	Yamashita *et al*., 2020; Luo *et al*., 2022
Diarrhea	4	26.7	Kudo 2018; Han *et al*., 2020
Elevated liver enzymes	3	20.0	Kayali *et al*., 2023; Wassmer *et al*., 2023
Skin rash	4	26.7	Han *et al*., 2020; Singh *et al*., 2021
Immune-mediated hepatitis	2	13.3	Kayali *et al*., 2023; Ju *et al*., 2023
Pneumonitis	1	6.7	Han *et al*., 2020; Wassmer *et al*., 2023
Acute graft rejection	1	6.7	Kayali *et al*., 2023; Wassmer *et al*., 2023
Grade ≥3 AEs	3	20.0	Yamashita *et al*., 2020; Kayali *et al*., 2023
Treatment discontinuation	1	6.7	Luo *et al*., 2022; Wassmer *et al*., 2023

Table 4: Radiological response per mRECIST criteria

Response type	No. of patients (n=15)	Percentage (%)
Complete response (CR)	2	13.3
Partial response (PR)	7	46.7
Stable disease (SD)	4	26.7
Progressive disease (PD)	2	13.3

Table 5: ctDNA levels at different timepoints

Timepoint	Mean ctDNA (copies/mL)	Standard deviation
Baseline	1140	250
6 Months	640	180
12 Months	340	95

Table 6: Tumor microenvironment markers pre- and post-therapy

Marker	Pre-treatment (mean ± SD)	Post-treatment (mean ± SD)
CD31 (vessel density)	45.6 ± 12.4	23.4 ± 8.9
MHC-I expression	Low	Moderate to High
PD-L1 tumor score	2.8 ± 0.5	1.5 ± 0.4
FoxP3+ tregs (cells/mm²)	58 ± 10.2	29 ± 7.8

Table 7: Comparative clinical outcomes of combination therapy versus monotherapy

Outcome	Lenvatinib + PD-L1 (Combination)	Lenvatinib alone	PD-(L)1 inhibitor alone	p-value*
Median OS(months)	36.0	14.2	11.3	0.018
Median progression-free survival (months)	12.0	6.1	4.5	0.006
ORR (ORR, %)	60.0	27.3	22.2	0.004
Disease control rate (DCR, %)	86.7	54.5	48.1	0.002
Tumor recurrence at 12 months (%)	20.0	41.7	46.2	0.031
Acute graft rejection (%)	6.7	0	23.1	0.041

*p-values derived from adjusted Cox regression or logistic regression analyses accounting for age, AFP, tumor burden, time since LT, and immunosuppressive regimen.

Table 8: Comparative immunological effects across treatment strategies

Immunological marker	Combination therapy	Lenvatinib alone	PD-(L)1 alone	p-interaction
$CD8^+$ T cells (cells/μL, Δ from baseline)	+200 ± 45	+65 ± 30	+95 ± 38	<0.001
Tregs (% change)	−4.7 ± 1.2	−1.4 ± 0.9	−2.1 ± 1.0	0.002
IFN-γ (pg/mL, Δ)	+23.4 ± 6.2	+6.8 ± 3.1	+11.9 ± 4.3	<0.001
TNF-α (pg/mL, Δ)	+12.1 ± 3.5	+3.9 ± 2.0	+6.4 ± 2.8	0.004
IL-10 (pg/mL, Δ)	−7.7 ± 2.1	−1.9 ± 1.0	−3.2 ± 1.4	0.003

Biomarker and tumor microenvironment analysis

Immunohistochemistry and ctDNA analysis revealed improved immunogenic tumor microenvironments. Increased infiltration of CD8+ T cells and reduced PD-L1 expression post-treatment supported the mechanistic benefit of combining angiogenesis inhibition with immune checkpoint blockade. A notable reduction in ctDNA levels further confirmed effective tumor burden reduction**.**

Radiological response per mRECIST criteria

In table 4, response to tumor is categorized at 12 months, according to the mRECIST criteria. A total of 2 patients (13.3%) responded completely and 7 patients (46.7%) responded partially, demonstrating that the combination therapy significantly decreased tumor mass. Of the four patients, two had SD and just two showed PD during treatment. The results demonstrate that 60% of patients had a measurable response, showing that using lenvatinib plus PD-L1 inhibition worked well for this group.

ctDNA Levels at different time points

At baseline and after 6 and 12 months, circulating tumor DNA (ctDNA) was tested to evaluate the molecular response. The mean ctDNA in the body decreased from 1140 copies/mL at the start to 340 copies/mL after 12 months (Table 5). It looks like the drug has helped curb tumor activity at the cellular level. The results agree with data from X-rays and suggest that measuring ctDNA can quickly monitor how well a cancer treatment is working.

Tumor microenvironment markers pre- and post-therapy

Described in table 6 are several important immunohistochemical markers found in the TME. The microvessel density marker CD31 decreased from 45.6 to 23.4 following treatment, indicating that lenvatinib achieved anti-angiogenic benefits. The change in major histocompatibility complex class I (MHC-I) expression from low to moderate/high indicated that tumor immunogenicity had improved. After PD-L1 treatment, the PD-L1 tumor score fell to 1.5 from 2.8 and the number of FoxP3+ Tregs was reduced by half, demonstrating that treating with PD-L1 made the immune response stronger.

Table 7 compares survival outcomes, tumor response, recurrence rates and graft safety among liver transplant recipients receiving combination therapy versus lenvatinib or PD-(L)1 inhibitor monotherapy. Combination therapy was associated with significantly prolonged overall and PFS, higher objective and DCRs and reduced tumor recurrence. Importantly, the incidence of acute graft rejection was lower than that observed with PD-(L)1 inhibitor monotherapy, indicating a favorable balance between oncologic efficacy and graft safety.

Table 8 summarizes treatment-associated changes in immune cell populations and cytokine profiles across combination therapy and monotherapy cohorts. Combination therapy resulted in significantly greater $CD8^+$ T-cell expansion, more pronounced suppression of Tregs, enhanced pro-inflammatory cytokine release and stronger reduction of immunosuppressive IL-10 levels. The p-interaction values indicate that immune modulation differed significantly between treatment strategies, supporting a synergistic immunological effect of angiogenesis inhibition combined with PD-L1 blockade.

Compared with lenvatinib or PD-(L)1 inhibitor monotherapy, combination therapy demonstrated superior survival outcomes, enhanced antitumor immune activation and a more favorable balance between efficacy and graft safety.

DISCUSSION

This study assessed how using lenvatinib with PD-L1 inhibition affects both treatment and the immune system in livers transplanted in patients with new or recently recurred HCC. Since the risk of rejection was previously a reason not to use immunotherapy after organ transplant, the results of this study suggest that combining immunotherapy with a strain immunosuppressive regimen may help treat cancers in this group with a favorable safety profile.

The survival benefit found in this study (36 months, p = 0.03) is similar to that seen with atezolizumab and bevacizumab used together in IMbrave150 for unresectable HCC, though for this trial, patients had not undergone transplants (Zhang *et al*., 2023). As per the data of this study, OS in transplanted patients was shorter than OS in patients not transplanted, but it matches the median OS of 10.5 months reported by other authors (Jin *et al*., 2022) in their work with post-LT patients receiving atezolizumab-bevacizumab. Importantly, this study can say that OS benefit resulted from the combined treatment with lenvatinib which has a strong action against VEGFR, FGFR and RET signaling, boosting the drug's anti-angiogenic capability. In this study, patients treated with both drugs had a PFS of 12 months which was significantly better than the standard therapy. The results from the SELECT trial are the same as those from the REFLECT trial which compared lenvatinib to sorafenib and found a PFS of 7.4 months for lenvatinib and 3.7 months for sorafenib (Kudo *et al*., 2022). This study extended PFS could indicate the benefits of immunotherapy in preventing tumor growth following transplant. The recorded rates of partial and complete response (46.7% and 13.3%) show how anti-angiogenic and immune checkpoint inhibition can kill tumor cells together.

Mechanistic insights into these outcomes are given through immunological markers. Following treatment, the number of CD8+ cells went up, as did the number of FoxP3+ Tregs and these patterns were maintained for the duration of the study. The transformation from an immunosuppressive to an immunoactive environment in the TME corresponds with other conducted studies (Lominadze *et al*., 2023) found that T-cell presence in HCC tumors helps predict ICIs response. Also, when PD-L1 expression decreases and IFN-γ and TNF-α increase, this indicates that immune exhaustion can be resolved and antitumor immunity can improve. Significantly, the ctDNA levels improved continuously from the start of treatment till 12 months, suggesting that treatment cleared the cancer at its source. The authors support this finding by pointing out that ctDNA is an effective tool for monitoring HCC growth. In the current study, a change in ctDNA kinetics strongly correlated with whether the tumor responded to treatment as shown on radiological scans.

Results from the study proved the therapy had acceptable safety, as this study did not notice major acute rejection after transplantation. In less than 20% of patients, major immune-related side effects appeared, compared to the 33% rejection rate by previous literature among patients using anti–PD-1. As a result, PD-L1 inhibitors have a potential advantage over PD-1 inhibitors in stopping graft rejection, owing to their limited effect in the tumor site and not throughout the body.

While isolated case reports have described the use of combined lenvatinib and immune checkpoint inhibition in liver transplant recipients with recurrent HCC, evidence at the cohort level remains extremely limited. Case reports primarily demonstrate individual clinical responses and do not allow systematic evaluation of survival outcomes, safety profiles, immune modulation, or biomarker dynamics (Jin *et al*., 2022). In contrast, the present study represents a cohort-based clinical and translational evaluation of this therapeutic strategy in post-transplant patients.

Despite the relatively small sample size, this study provides several novel contributions. First, it offers systematic survival data, including overall survival, PFS and ORRs, rather than anecdotal outcomes. Second, it integrates comprehensive immunological profiling, demonstrating increased intratumoral $CD8^+$ T-cell infiltration, reduced regulatory T-cell populations and favorable cytokine shifts following combination therapy. Third, this study incorporates circulating tumor DNA (ctDNA) dynamics as a molecular marker of treatment response, providing early insight into tumor burden reduction beyond radiological assessment. Finally, detailed evaluation of graft safety and irAEs suggests that PD-L1 inhibition, when combined with anti-angiogenic therapy under controlled immunosuppression, may be feasible in selected liver transplant recipients.

Although limited by its retrospective design and modest sample size, the study extends existing case-based observations by offering biological, molecular and clinical validation at the cohort level. These findings strengthen the rationale for combination therapy in this high-risk population and provide a foundation for future multicenter prospective trials. Importantly, the consistency of clinical benefit across survival, immune activation and molecular response endpoints supports the translational relevance of this approach despite the inherent limitations of patient numbers in post-transplant oncology studies.

Lenvatinib clearly reduced the number of microvessels seen on CD31 staining following treatment. The elevated MHC-I expression observed in tumors after treatment explains part of the role of ICI drugs, as per other authors (General *et al*., 2023) explanation ICI success relies on immune responses. Even though the findings are

promising, this study's limited size and design at just one center limits its scope. It is still unknown how safe these treatments continue to be after 18 months and when they should be given gently after transplant (ICH, 2001; Kayali *et al*., 2023). Moreover, the best way to take immunosuppressants together with immunotherapy needs to be studied, because interaction between drugs may affect the effectiveness and survival of the transplant. In brief, this investigation expands the evidence showing that combining immune checkpoint inhibition and targeted drugs like lenvatinib is efficient and safe for liver transplant recipients with HCC. Not only the results of this study suggest a useful treatment method for this high-risk patient group, but they also allow for encouraging bigger, multicenter prospective studies to justify the findings and form clearer criteria for choosing patients.

Clinical outcomes observed in this study are now interpreted in the context of prior evidence from the REFLECT trial, IMbrave150 and emerging post-transplant immunotherapy reports. The observed improvement in OSand PFS aligns with prior data demonstrating enhanced efficacy of anti-angiogenic therapy when combined with immune checkpoint inhibition in advanced HCC.

Immunological findings, including increased $CD8^+$ T-cell infiltration, reduced regulatory T-cell populations and favorable cytokine modulation, are discussed in relation to published studies describing tumor microenvironment reprogramming by lenvatinib and PD-L1 blockade. The reduction in ctDNA levels is interpreted as molecular confirmation of tumor burden reduction, consistent with emerging literature supporting ctDNA as a sensitive biomarker for treatment response in HCC.

Safety outcomes are contextualized against previously reported rejection rates with PD-1 inhibitors in transplant recipients, highlighting the potential relative safety of PD-L1 inhibition under controlled immunosuppression. These comparisons strengthen the biological plausibility and clinical relevance of the findings.

This study has several notable strengths. It represents one of the few cohort-level evaluations of combined lenvatinib and PD-L1 inhibition in liver transplant recipients with recurrent or de novo HCC. The integration of clinical outcomes with immunological profiling and molecular biomarkers provides a comprehensive translational perspective. The inclusion of ctDNA analysis offers an objective, non-invasive marker of tumor response that complements radiological assessment. Additionally, the systematic evaluation of graft safety and irAEs contributes valuable data to an area where clinical evidence remains limited. Together, these strengths enhance the scientific and clinical relevance of the study despite inherent limitations related to sample size.

CONCLUSION

In this cohort-based analysis of liver transplant recipients with recurrent or de novo HCC, combination therapy with lenvatinib and PD-L1 inhibition demonstrated superior clinical efficacy compared with either lenvatinib or PD-(L)1 inhibitor monotherapy. Patients receiving combination treatment experienced prolonged OSand PFS, higher objective and DCRs and reduced tumor recurrence. These clinical benefits were accompanied by enhanced tumor immunogenicity, characterized by increased $CD8^+$ T-cell infiltration, suppression of regulatory T-cell populations, favorable cytokine modulation and sustained reductions in circulating tumor DNA levels.

Importantly, combination therapy did not result in a disproportionate increase in severe irAEs or acute graft rejection when administered under carefully controlled immunosuppression. Compared with PD-(L)1 inhibitor monotherapy, the combination approach appeared to maintain a more favorable balance between antitumor immune activation and graft tolerance, supporting its feasibility in selected post-transplant patients.

Although limited by its retrospective design and modest sample size, this study provides the first cohort-level evidence demonstrating the added clinical and immunological value of combining angiogenesis inhibition with PD-L1 blockade over monotherapy in liver transplant recipients. These findings support further prospective, multicenter studies to validate efficacy, optimize immunosuppressive strategies and define patient selection criteria for combination therapy in post-transplant HCC.

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None.

Authors' contributions
Chao Ma and Chaolu Li: Developed and planned the study, performed experiments and interpreted results. Edited and refined the manuscript with a focus on critical intellectual contributions; Haitao Zhang and Junfeng Lu: Participated in collecting, assessing and interpreting the data. Made significant contributions to data interpretation and manuscript preparation; Qikun Zhang: Provided substantial intellectual input during the drafting and revision of the manuscript.

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Data availability statement
All the data has been included in the study

Ethics approval
This study was conducted in accordance with the principles of the Declaration of Helsinki and was approved by the Ethics Committee of Beijing You'an Hospital, Capital

Medical University [Approval No. (2024)080]. This study was performed in adherence with the STROBE guidelines. See supplementary file for the STROBE checklist.

Conflict of interest

The authors declare that they have no financial conflicts of interest.

Consent to participate

Informed consent was obtained from all participants prior to inclusion in the study.

Supplementary data

https://www.pjps.pk/uploads/2026/05/SUP1779791353.pdf

REFERENCES

Abdelhamed W and M El-Kassas (2023). HCC recurrence: Predictors and management. *Liver Res.*, **7**(4): 321-332.

Ahn, KS, O'Brien DR., Kim YH., Kim TS., Yamada H, Park JW, Park SJ, Kim SH, Zhang C, Li H, Kang KJ and Roberts LR (2021). Associations of serum tumor biomarkers with integrated genomic and clinical characteristics of HCC. *Liver cancer*, **10**(6): 593-605.

Chen L, Song M and Yao C (2021). Calcineurin in development and disease. *Genes Dis*., **9**(4): 915-927.

Cucchetti A, Elshaarawy O, Han G, Chong CCN, Serra C, O'Rourke JM, Crew R, Felicani C, Ercolani G, Shah T, Vogel A, La PBS and Johnson PJ (2023). 'Potentially curative therapies' for HCC: How many patients can actually be cured?. *Br J Cancer*, **128**(9): 1665-1671.

Gao YX, Ning QQ, Yang PX, Guan YY, Liu PX, Liu ML, Qiao LX, Guo XH, Yang TW and Chen DX (2023). Recent advances in recurrent HCC therapy. *World J Hepatol*., **15**(4): 460-476.

General Assembly of the World Medical Association (2014). World medical association declaration of Helsinki: Ethical principles for medical research involving human subjects. *J Am Coll Dent*., **81**(3): 14-18.

Han Y, Liu D and Li L (2020). PD-1/PD-L1 pathway: Current researches in cancer. *Am J Cancer Res.*, **10**(3): 727-742.

International conference on Harmonisation of technical requirements for registration of pharmaceuticals for human use. (2001). ICH harmonized tripartite guideline: Guideline for good clinical practice. *J Postgrad Med*., **47**(1): 45-50.

Jain P, Shasthry S M, Choudhury A K, Maiwall R, Kumar G, Bharadwaj A, Arora V, Vijayaraghavan R, Jindal A, Sharma M K, Bhatia V and Sarin SK (2021). Alcohol associated liver cirrhotics have higher mortality after index hospitalization: Long-term data of 5,138 patients. *Clin Mol Hepatol.*, **27**(1): 175-185.

Jin X, Zhang K, Fang T, Zeng X, Yan X, Tang J, Liang Z, Xie L and Zhao D (2022). Low-dose PD-1 inhibitor combined with lenvatinib for preemptive treatment of recurrence after LT for HCC: Case report and literature review. *Front Oncol*., 2022. **12**: 951303.

Ju F, Wang D, Huang L, Jiang C, Gao C, Xiong C and Zhai G (2023). Progress of PD-1/PD-L1 signaling in immune response to LT for HCC. *Front Immunol.*, **14**: 1227756.

Kayali S, Pasta A, Plaz Torres MC, Jaffe A, Strazzabosco M, Marenco S and Giannini EG (2023). Immune checkpoint inhibitors in malignancies after LT: A systematic review and pooled analysis. *Liver Int*., **43**(1): 8-17.

Kudo M (2018). Lenvatinib may drastically change the treatment landscape of HCC. *Liver Cancer*, **7**(1): 1-19.

Li Q, Han J, Yang Y and Chen Y (2022). PD-1/PD-L1 checkpoint inhibitors in advanced HCC immunotherapy. *Front Immunol*., **13**: 1070961.

Liu H, Sethi V, Li X, Xiao Y and Humar A (2024). LT for HCC: A narrative review and a glimpse into the future. *Semin Liver Dis*., **44**(1): 79-98.

Liu YB, Chen MK (2022). Epidemiology of liver cirrhosis and associated complications: Current knowledge and future directions. *World J Gastroenterol*., **28**(41): 5910-5930.

Lominadze Z, Hill K, Shaik MR, Canakis JP, Bourmaf M, Adams-Mardi C, Abutaleb A, Mishra L and Shetty K (2023). Immunotherapy for HCC in the setting of LT: A review. *Int J Mol Sci.*, **24**(3): 2358.

Luo J, Gao B, Lin Z, Fan H, Ma W, Yu D, Yang Q, Tian J, Yang X and Li B (2022). Efficacy and safety of lenvatinib versus sorafenib in first-line treatment of advanced HCC: A meta-analysis. *Front Oncol*., **12**: 1010726.

Ma L, Hernandez MO, Zhao Y, Mehta M, Tran B, Kelly M, Rae Z, Hernandez JM, Davis JL, Martin SP, Kleiner DE, Hewitt SM, Ylaya K, Wood BJ, Greten TF and Wang XW (2019). Tumor cell biodiversity drives microenvironmental reprogramming in liver cancer. *Cancer Cell*., **36**(4): 418-430 e6.

Matsuki M, Hoshi T, Yamamoto Y, Ikemori-Kawada M, Minoshima Y, Funahashi Y and Matsui J (2018). Lenvatinib inhibits angiogenesis and tumor fibroblast growth factor signaling pathways in human HCC models. *Cancer Med*., **7**(6): 2641-2653.

Mazzaferro V, Bhoori S, Sposito C, Bongini M, Langer M, Miceli R, Mariani L (2011). Milan criteria in LT for HCC: An evidence-based analysis of 15 years of experience. *Liver Transpl.*, **17**(Suppl 2): S44-57.

Panackel C, Mathew JF, Fawas N M, Jacob M (2022). Immunosuppressive drugs in liver transplant: An insight. *J Clin Exp Hepatol.,* **12**(6): 1557-1571.

Pinero F, Thompson M, Marin JI and Silva M (2020). Lenvatinib as first-line therapy for recurrent HCC after LT: Is the current evidence applicable to these patients? *World J Transplant*., **10**(11): 297-306.

Puri P and Malik S (2023). LT: Contraindication and ineligibility. *J Clin Exp Hepatol.*, **13**(6): 1116-1129.

Singh V, Khurana A, Allawadhi P, Banothu AK, Bharani KK, Weiskirchen R (2021). Emerging role of PD-1/PD-

L1 inhibitors in chronic liver diseases. *Front Pharmacol.*, **12**: 790963.

Spahn S, Kleinhenz F, Shevchenko E, Stahl A, Rasen Y, Geisler C, Ruhm K, Klaumuenzer M, Kronenberger T, Laufer SA, Sundberg-Malek H, Bui KC, Horger M, Biskup S, Schulze-Osthoff K, Templin M, Malek NP, Poso A and Bitzer M (2024). The molecular interaction pattern of lenvatinib enables inhibition of wild-type or kinase-mutated FGFR2-driven cholangiocarcinoma. *Nat Commun*., **15**(1): 1287.

Sposito C, Citterio D, Virdis M, Battiston C, Droz Dit Busset M, Flores M, Mazzaferro V (2022). Therapeutic strategies for post-transplant recurrence of HCC. *World J Gastroenterol*., **28**(34): 4929-4942.

Terrault NA, Francoz C, Berenguer M, Charlton M and Heimbach J (2023). LT 2023: Status report, current and future challenges. *Clin Gastroenterol Hepatol*., **21**(8): 2150-2166.

Wassmer CH, El Hajji S, Papazarkadas X, Compagnon P, Tabrizian P, Lacotte S and Toso C (2023). Immunotherapy and LT: A narrative review of basic and clinical data. *Cancers (Basel)*, **15**(18): 4574.

Wheler JJ, Atkins JT, Janku F, Moulder SL, Stephens PJ, Yelensky R, Valero V, Miller V, Kurzrock R and Meric-Bernstam F (2016). Presence of both alterations in FGFR/FGF and PI3K/AKT/mTOR confer improved outcomes for patients with metastatic breast cancer treated with PI3K/AKT/mTOR inhibitors. *Oncoscience*, **3**(5-6): 164-172.

Zhang X, Cai H, Peng W, Wang H, Wu J, Zhu X, Guo W, Xie F, Zhang Y, Wang M and Yu Y (2026). Lenvatinib plus transarterial chemoembolization and PD-1 inhibitors as conversion therapies for unresectable intermediate-advanced HCC: A phase 2 trial and exploratory biomolecular study. *Sig Transduct Target Ther* **11**(1): 37.

Yamashita T, Kudo M, Ikeda K, Izumi N, Tateishi R, Ikeda M, Aikata H, Kawaguchi Y, Wada Y, Numata K, Inaba Y, Kuromatsu R, Kobayashi M, Okusaka T, Tamai T, Kitamura C, Saito K, Haruna K, Okita K and Kumada H (2020). REFLECT-a phase 3 trial comparing efficacy and safety of lenvatinib to sorafenib for the treatment of unresectable HCC: An analysis of Japanese subset. *J Gastroenterol*., **55**(1):113-122.

Zhang TQ, Geng ZJ, Zuo MX, Li JB, Huang JH, Huang ZL, Wu PH and Gu YK (2023). Camrelizumab (a PD-1 inhibitor) plus apatinib (an VEGFR-2 inhibitor) and hepatic artery infusion chemotherapy for HCC in barcelona clinic liver cancer stage C (TRIPLET): A phase II study. *Signal Transduct Target Ther*., **8**(1): 413.

Zou J, Huang P, Ge N, Xu X, Wang Y, Zhang L, Chen Y (2022). Anti-PD-1 antibodies plus lenvatinib in patients with unresectable HCC who progressed on lenvatinib: A retrospective cohort study of real-world patients. *J Gastrointest Oncol.*, **13**(4): 1898-1906.