STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No.	Recommendation	Page No.	Relevant text from manuscript
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1	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.
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	1	Background: Long-term immunosuppression from transplants which reduces the body's ability to detect and fight the tumor, is the main reason 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 liver transplantation. 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 OS, PFS, ORR, instances of tumor recurrence and events of adverse reactions related to treatment. Flow cytometry, ELISA and immunohistochemistry were used to estimate the presence of CD8+ T cells, regulatory T cells

				(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 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.
Introduction				
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	2-3	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, corticosteroids and antimetabolites are standard therapies, yet they may facilitate tumor recurrence. HCC remains a leading indication for LT, particularly in 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 AFP and DCP (Liu et al., 2024). Immune checkpoint inhibitors (ICIs) have transformed HCC therapy but pose substantial risk post-transplant, as 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).
Objectives	3	State specific objectives, including any prespecified hypotheses	3	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.
Methods				
Study design	4	Present key elements of study design early in the paper	3	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 hepatocellular carcinoma (HCC) or de novo hepatic malignancies.
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	3	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.
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	3	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.
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case	3	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.
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	3	Both the main endpoints for this study were overall survival (OS) which means the years from the beginning of treatment to death or the last time the subjects

				were checked and progression-free survival (PFS) which is the period until disease worsening or death.
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	3-4	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 ctDNA levels were determined in digital PCR at three time points: from the start, after 6 months and at 12 months after treatment.
Bias	9	Describe any efforts to address potential sources of bias	4	Comparative analyses were adjusted for baseline age, time since transplantation, tumor burden, AFP level and immunosuppressive regimen.
Study size	10	Explain how the study size was arrived at	4	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.

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Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	4	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.
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	4	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.
		(*b*) Describe any methods used to examine subgroups and interactions	4	Relationships between immunological markers and treatment success were evaluated using Pearson or Spearman correlation coefficients.
		(*c*) Explain how missing data were addressed	4	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.
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy	4	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.
		(*e*) Describe any sensitivity analyses	4	A logistic regression analysis was carried out to see which factors might be tied to higher-grade side effects or treatment interruption.
Results				
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	4	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 liver transplantation 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.
		(b) Give reasons for non-participation at each stage	4	Patients were selected using identical inclusion and exclusion criteria as the combination cohort.
		(c) Consider use of a flow diagram	-	-
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	4	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 liver transplantation 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.
		(b) Indicate number of participants with missing data for each variable of interest	-	-
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	7	Median overall survival (months) 36.0; Median progression-free survival (months) 12.0.
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	4	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%).
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	-	-
		Cross-sectional study—Report numbers of outcome events or summary measures	-	-

Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	7	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 progression-free survival, higher objective and disease control rates 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.
		(*b*) Report category boundaries when continuous variables were categorized	4	The tumor response was determined using mRECIST v1.1 and given as CR, PR, SD or PD.
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	-	-

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Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	5-8	Immunological profile, Biomarker and tumor microenvironment analysis, Radiological response per mRECIST criteria, ctDNA Levels at different time points, Tumor microenvironment markers pre- and post-therapy, Comparative immunological effects across treatment strategies.
Discussion				
Key results	18	Summarise key results with reference to study objectives	8	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.
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	8-9	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).
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	8-9	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 hepatocellular carcinoma, 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, progression-free survival and objective response rates, 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 immune-related adverse events suggests that PD-L1 inhibition, when combined with anti-angiogenic therapy under controlled immunosuppression, may be feasible in selected liver transplant recipients. 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 overall survival and progression-free survival 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 hepatocellular carcinoma. 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 immune-related adverse events 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.
Generalisability	21	Discuss the generalisability (external validity) of the study results	9	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 hepatocellular carcinoma.
Other information				
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	10	Funding There was no funding.

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.