nature portfolio

Peer Review File

The anti-PD-L1/CTLA-4 bispecific antibody KN046 plus lenvatinib in advanced unresectable or metastatic hepatocellular carcinoma: a phase II trial

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This file contains all reviewer reports in order by version, followed by all author rebuttals in order by version.

Version 1:

Reviewer comments:

Reviewer #1

(Remarks to the Author)
I have read with great interest the work from Xu et al., and I would like to congratulate the authors for their effort. They illustrate the promising results of a prospective clinical trial investigating a compound with a novel mechanism of action. The combination of the bispecific antibody against PD-L1/CTLA4 and the oral TKI lenvatinib achieved a remarkable ORR of 45.5%, making this combination an appealing treatment strategy to be tested in randomised clinical trials. The clinical findings are nicely complemented by the translational analyses on ctDNA, whose clearance was found to be associated with long-term survival.
I believe the authors might consider some modifications to their manuscript, in order to improve the overall quality of their work.

Please find below my specific comments:
• Abstract: I would suggest including the centres where the patients were enrolled and the data cutoff for the analyses. If I understand correctly from the rest of the manuscript, the median OS was calculated with a data cut off in 2024, while median PFS was mature at a previous interim analysis. I believe methodologically this should be revised, and all the results should be presented with the same data cutoff. Also, I would improve the flow of the paragraph on TRAEs and Grade 3 AEs, as it is not completely clear. I would also present all results with the time calculated in months and not in weeks.
• Background
I believe the introduction can be improved by introducing more references to the current literature.
o Line 63-63: median OS exceeds 22 months in the most recent phase 3 trials (for example, checkmate 9DW), so this should be amended
o If the authors want to mention the mechanism of action of immune checkpoint inhibitors, I would appreciate more detail, as “promoting the destruction of tumour cells” can sound simplistic to the readers of Nature.
o The authors should include updated references to the Phase 3 studies in the field. For example, the Checkmate 9DW tested the double ICI combination of nivo-ipi, or the LEAP 002 (should be added to line 80)
o Line 84: dual immunotherapy might significantly improve patient survival compared to single agent ICI. This is not true, as the HIMALAYA trial showed. Please reconsider this part.
o Maybe the authors want to mention the rationale of combining double ICI with Lenvatinib, and maybe mention the reason why they performed the ctDNA analysis (previous data in this setting?)

• Methods
o I believe that the Methods could seem a bit redundant, as most of this information are present in the Study protocol that should be available in the supplementary material.
o A list of the “multi-centers” (line 108, please find an alternative definition) involved in the trial should be provided
o Line 118: only histological diagnosis is listed. Could the authors explain if radiological diagnosis according to AASLD criteria was allowed? If not, could they confirm that all patients have an available tumour biopsy?
o Line 121: systematic is a typo, as it should be systemic
o Line 123: fibrous lamina -> fibrolamellar?
o Line 146: please use months as measure of time
o If I understand correctly, the bispecific Ab was administered up to 2 years, while Lenvatinib was allowed even beyond that

until disease progression. Could the authors explain the rationale behind this choice?
• Results
o Data cutoff: this is a key point to clarify. It seems like there was a data cutoff for most of the analyses (line 261: November 30, 2022) and one for OS (Line 298: March 15, 2024). I believe all the analyses should be presented with a univocal data cutoff.
o I do not fully understand the reason to present the results according to the dose of Lenvatinib. Lenvatinib is administered at 8 or 12mg according to patients' weight as per summary of product characteristics, but this does not impact efficacy or safety. Many oncological medications are administered at different doses depending on the BSA of the patients, or their weight. I would suggest presenting all the results (including the tables) regardless of Lenvatinib dose, otherwise it might give the false impression that these are two different arms with two different expected outcomes.
o The authors should describe in detail the time of previous anticancer therapy (table 1). Inclusion criteria allowed only patients without any previous systemic therapy. Could the authors clarify?
o In the safety table, I would strongly recommend reporting the overall adverse events, regardless of the attribution of causality to the treatment.
o In the baseline characteristics table, the authors list around 27% of patients with non-viral aetiology. Could they add some further detail, for instance re: MASLD diagnosis? Also, how was cirrhosis diagnosed?
o Could the authors explain what they mean with "duration of HCC" in table 1?
o In Figure 1, what do they mean as "investigators' judgement" as cause of treatment discontinuation?
o Kaplan meier curves: minor typo "Number of risk" instead of "at risk"
o In the ctDNA analysis, did the authors identify any difference in ctDNA clearance across responders (CR+PR) vs non-responders?
• Discussion
o I would use caution when describing the safety profile of this combination all across the manuscript. For instance (Line 333) I am not sure it is fully correct to say that the safety profile was manageable, as there is no clear definition of "manageable". 42% of G3-5 trAE is not negligible, and the authors should reinforce this issue.
o The combination of Lenvatinib-pembrolizumab in the Phase III LEAP 002 trial caused 62% of G3-G4 trAEs. The authors should explain why they think their compound (which also includes an antiCTLA4, notably associated with high rate of immune-related AE) causes a lower rate of AEs, even when combined with Lenvatinib which, alone, was showed to be associated with >50% G3-4 trAE (LEAP 002 control arm Lancet Oncol 2023; REFLECT Lancet 2018). They will need to elaborate on this point further, and consider restructuring the discussion to highlight the toxicity of the combination. Please consider rephrasing line 381 "..were acceptable", as there is no consensus on what "acceptable" should be.
o Line 354: please add the correct reference to the HIMALAYA trial
o Lines 383-385: I am not sure this is in line with current literature. Durva-treme in the HIMALAYA trial were associated with 25.8% of G3-4 trAEs, way less than the combination tested in this manuscript. Please consider restructuring the discussion accordingly.
o I would suggest expanding more the limitations of the study, including that the population enrolled has a univocal geographical origin, and so the conclusions from this study cannot be generalised (for instance, due to the majority of HBV-associated HCC).

Reviewer #2

(Remarks to the Author)
Xu et al. report the results of a phase II clinical study on the efficacy and safety of KN046, a novel bispecific antibody targeting programmed death ligand 1 (PD-L1) and cytotoxic T lymphocyte-associated protein 4 (CTLA-4) combined with lenvatinib in patients with advanced HCC. Moreover, the goal was to explore potential dynamic biomarkers using next-generation sequencing (NGS). The results showed acceptable adverse events and a promising anti-tumor effect of combination treatment using dual immune checkpoint inhibition with lenvatinib. This study is interesting, novel, and rigorously designed, and the results are clinically relevant. There are several concerns and areas of improvement in data presentation, as detailed below.
1. Lines 94-97: The authors discuss their previous findings in the Introduction (reported in Jiang et al., Adv Sci, 2021: 2102500) as a motivation for this study. However, that study reported that intra-tumoral injection of KN046 in mice can be safely and effectively delivered by the ZIF-8 delivery system. Since the delivery route was different, it is difficult to determine the relevance of those findings for this clinical study.
2. On page 8, Lines 194-195, the description of the time points used for ctDNA collection is unclear. Please clarify when p3 is in the methods. Are p2 and p3 the same time point?
3. On page 22, the description of the Clinical Study Protocol KN046-IST-05: Why were dose adjustments of KN046 not allowed? What was the management for the patients with AE grade>3 during the period of treatment interruption?
4. Figure 4: The analysis of RNA sequencing data for the immune classification of HCC (Kurebayashi et al., Hepatology. 2022; 75(5): 1139-1153) would be useful to increase the utility of the biomarker.
5. Line 362-365, 387-390: The previous papers have indicated KN046 has promising antitumor efficacy. Please discuss the mechanisms of potential synergy (and toxicity) between lenvatinib and KN046.
6. Based on the ctDNA biomarker analysis results at the pre-treatment time point (P0), can the authors discuss more clearly which genetic variations detected by this non-invasive method might predict a patient's non-responsiveness? This could help identify patients who are more likely to respond to the treatment before its administration (as a predictive biomarker). This will be more useful than a prognostic biomarker (which defines the outcomes irrespective of treatment). Moreover, a randomized study will be required to determine whether the dynamic biomarker is prognostic or predictive.
7. The manuscript would benefit from editing for English.

Reviewer #3

(Remarks to the Author)
This study was an Phase II study designed to assess the effectiveness and safety profile of KN046, an innovative bi-specific antibody directed against both programmed death ligand 1 (PD-L1) and cytotoxic T lymphocyte-associated protein 4 (CTLA-4), when administered in conjunction with Lenvatinib for the treatment of advanced hepatocellular carcinoma (HCC). Additionally, the investigation aimed to uncover potential biomarkers that could further inform the use of this therapeutic combination.
The results showed that the combination treatment regimen achieved positive results in tumor response and survival. In addition, the study analyzed the relationship between genetic characteristics and gene variation of tumor tissue and treatment response through next-generation sequencing, as well as the relationship between the positive rate of circulating tumor DNA and progressive survival.
The noteworthy findings highlight several key points:
1. Promising Potential of Bispecific Antibodies in HCC Immunotherapy: The study revealed that the proportion of patients achieving durable clinical responses with the bispecific antibody KN046 surpassed that achieved with separate antibodies targeting the PD-1/PD-L1 and CTLA-4/B7 pathways. Notably, the observed overall response rate (ORR) of 46% exceeded the 13%-30% range reported in previous clinical trials, suggesting a more robust therapeutic effect. Furthermore, patients experienced a longer median overall survival (mOS) of 16.4 months, indicating a substantial survival benefit.
2. Favorable Safety Profile and Convenience: KN046, as a bispecific antibody, demonstrated fewer side effects compared to the administration of two separate PD-1/L1 and CTLA-4 antibodies. This favorable safety profile allowed for a longer duration of treatment, resulting in better tumor control. The convenience of KN046 administration, requiring only a single infusion, further simplifies the treatment regimen and enhances patient compliance.
3. Biomarker Identification: Dynamic changes in ctDNA can be used as potential biomarkers to predict patient response to treatment and survival. These results suggest that monitoring changes in ctDNA can help assess patients' treatment response and predict their survival, providing an important basis for individualized treatment decisions.
4. Pioneering Treatment Option: These results firmly position the KN046 plus lenvatinib combination as a potential new frontier in the treatment landscape for advanced HCC. This is the first exploratory study to evaluate not only a bispecific antibody but also its combination with a small molecular tyrosine kinase inhibitor (TKI) in the context of HCC, marking a significant advancement in the field.

In summary, the study exhibits rigorous design, employs reasonable research methodologies, demonstrates high execution standards, and presents reliable conclusions. Nevertheless, there are a few questions that I would like to pose to the authors for clarification.
1、In this study，“TRAEs occurred in 100% of the participants, of which grade ≥3 occurred in 26 (47.3%).” “Thirteen (23.6%) participants experienced irAEs.” In the table 2，we can conclude that 54 (98.2%) cases suffered from TRAE and 15 (27.3%) cases Grade ≥3. Regarding the drug KN046, it is noteworthy that the incidence of adverse reactions is indeed very low, which is quite contrary to our previous impressions of CTLA-4 antibodies or bispecific antibodies. This raises the question of whether the researchers employed any specific strategies for controlling or preventing adverse reactions?
2. We are concerned that in this study, a significant number of patients received 12mg Lenvatinib qd, so what are the similarities and differences in the incidence of Lenvatinib-related adverse reactions among patients in different dose groups? What are the differences in effectiveness between the two groups?
3. In this study, 44 patients retained baseline tissue samples. Plasma was available for 50 patients at some point in time. We understand that this is a very large amount of work, showcasing the dedication and thoroughness of the researchers. Specifically, we seek clarity on the protocols employed for acquiring these tissue specimens, as their acquisition is paramount to the integrity and validity of the research findings. Additionally, we are concerned about any potential adverse reactions or complications associated with these procedures, as we prioritize patient safety above all else.
4. We have noted that baseline tissue specimens were retained in this study, which prompts us to inquire about the detection strategy for ctDNA: is it tumor-informed or tumor-agnostic? This question arises particularly because we also observe that the number of patients from whom blood samples were collected exceeds the number of tissue specimens.
5. In this study, 54.5% of the patients did not achieve an objective response. May we inquire whether these patients received any corresponding subsequent line of treatment? Furthermore, do the researchers believe that these subsequent treatments had any impact on the patients' survival?
6. Have the researchers continued to follow up on the long-term survival data of this cohort? The potential long-term survival benefits of this cohort could be attractive. Of course, the case number in a Phase II study is limited, and its conclusions need further validation. Hence, we eagerly anticipate the corresponding Phase III study. Do the researchers have any plans for such a study?

Reviewer #4

(Remarks to the Author)
Congratulations to the authors for designing and conducting this interesting phase II clinical trial that investigates the safety and efficacy of KN046 in combination with lenvatinib in advanced unresectable or metastatic hepatocellular carcinoma. This is a clinically meaningful study and would provide important insights on the efficacy of this combination treatment, Overall, the paper was well-written and could be strengthened with clearer presentation of the results.
Comments
1. Abstract Line 49: Could the author confirm what circulating tumour DNA status at baseline are they referring to? Based on Figure S2C, P0 was not associated with either PFS or OS.
2. Endpoints Line173-175, could the authors include information on how censorings were handled for PFS and OS; when are patients censored for these endpoints.
3. Discussion Line 358-360, the median PFS was 11.0 (95% CI: 8.21-15.24) months, it would not be correct to state that the

median PFS exceeded 11.0 months.
4. Please include in the statistical analysis section what method was used to compute the median follow up.
5. Please include in the statistical analysis section what method was used to compute the hazard ratios reported.
6. Table 1. For Duration of HCC and total diameter of target lesions (RECIST and mRECIST), could the authors please clarify what are presented? Do they represent mean with standard deviation, median with interquartile range or range or others?

Reviewer #5

(Remarks to the Author)
“I co-reviewed this manuscript with one of the reviewers who provided the listed reports. This is part of the Nature Communications initiative to facilitate training in peer review and to provide appropriate recognition for Early Career Researchers who co-review manuscripts.

Version 2:

Reviewer comments:

Reviewer #1

(Remarks to the Author)
I would like to thank the authors for addressing the reviewers' comments. By doing so, I believe their manuscript has remarkably improved.
I would like to reinforce the importance of describing the HCC aetiology with more granularity. In any manuscript about HCC, aetiology is reported as being related to HBV, HCV, or non-viral. The non-viral category encompasses not only metabolic-related HCC (such as MASLD), but also patients with HCC due to alcohol consumption or due to an unknown cause. I believe the authors should clarify whether the 27% patients with "other aetiology" (Table 1) were all diagnosed with MASLD. I would recommend breaking down the "Others" aetiology (Table 1) into metabolic liver disease (MASLD), alcohol, and unknown, in line with the reporting practice in the current literature.

Reviewer #2

(Remarks to the Author)
The authors have revised the manuscript and have addressed appropriately all my concerns.

Reviewer #3

(Remarks to the Author)
The authors have provided satisfactory responses to the questions raised in our review comments and supplemented the paper with corresponding detailed information. We acknowledge the efforts made by the author team. As before, we believe that this is a meaningful clinical study with research results that are highly relevant to clinical practice. There are still some parts of the article that require careful polishing by English professionals. In our opinion, the article should be accepted for publication.

Reviewer #4

(Remarks to the Author)
The authors have satisfactorily addressed my comments, except for comments 2 and 5.

With regards to comment #2, could the author confirm if the censoring information for PFS and OS are included in the manuscript or the accompanying protocol/statistical analysis plan/supplementary materials?

With regards to comment #5, the HRs reported in the previous manuscript were not related to the drug dosing, hence, the removal of dosage cohorts would not render it unnecessary to define the method used to estimate the HRs reported in this manuscript. HRs were reported in Figure 4(D and E) and Supplementary Figure 3 (C-E) of the revised manuscript.

Given the change in the data used for the overall survival analysis, I have an additional comment. Please note that the median OS was estimated as 16.4 months, while the upper limit of the 95% CI for the median OS is “not estimable” instead of “not reached”. This is presented correctly in Figure 3C but not in the main manuscript text.

Reviewer #5

(Remarks to the Author)
I co-reviewed this manuscript with one of the reviewers who provided the listed reports. This is part of the Nature Communications initiative to facilitate training in peer review and to provide appropriate recognition for Early Career Researchers who co-review manuscripts.

Version 3:

Reviewer comments:

Reviewer #4

(Remarks to the Author)
The authors have addressed all my concerns and revised the manuscript to improve the presentation of their findings.

Reviewer #1 (Remarks to the Author): with expertise in HCC, therapy

Abstract:

- *I would suggest including the centres where the patients were enrolled and the data cutoff for the analyses.*

Response: We appreciate the Reviewer's suggestion to provide additional information regarding the enrollment centers and the data cut-off date. Initially, we had anticipated the participation of three centers: Beijing Cancer Hospital, Harbin Medical University Cancer Hospital, and Shenzhen Nanshan People's Hospital. However, due to the absence of eligible patients at the Shenzhen site, only two centers were ultimately involved. Additionally, we have unified the data cut-off date to November 30th, and this has been incorporated into the abstract for clarity.

The following is revised in the Abstract section:

Methods: This open-label phase 2 trial enrolled treatment-naïve patients with advanced HCC from September 2020 to October 2021 at two centers in China.

Results: At the cut-off date of November 30, 2022, fifty-five participants were enrolled; median exposure for KN046 and lenvatinib was 7.1 (0.7-23.4) months and 7.7 (0.3-25.0) months, respectively.

The following is revised in the Methods section:

This multi-center open-label phase 2 clinical trial enrolled patients with advanced HCC from September 2020 to October 2021, at Beijing Cancer Hospital and Harbin Medical University Cancer Hospital in China.

- *If I understand correctly from the rest of the manuscript, the median OS was calculated with a data cut off in 2024, while median PFS was mature at a previous interim analysis. I believe methodologically this should be revised, and all the results should be presented with the same data cutoff.*

Response: We appreciate the insightful feedback and suggestions provided. We

completely agree that for consistency and methodological accuracy, all results should be presented using the same data cut-off. In response to your recommendation, we have revised the data for both overall survival (OS) and progression-free survival (PFS) in our manuscript. All results are now based on a unified data cut-off date of November 30, 2022. This adjustment has led to an update in the median OS, which is now reflected as 16.4 months (95% CI, 11.20-NR) in the Abstract and Results sections of the manuscript. Furthermore, the Kaplan-Meier curve for OS, previously Figure 3C, has been replaced with a new figure that accurately represents the data up to the new cut-off date.

The following is revised in the Abstract Section:

Results: The median overall survival (OS) was 16.4 (95% CI, 11.20-not reached) months, and 12-month OS rate was 60.0% (95% CI, 45.87-71.55).

- *I would improve the flow of the paragraph on TRAEs and Grade 3 AEs, as it is not completely clear.*

Response: We appreciate your feedback regarding the clarity of Abstract section. In response to your suggestion, we have made improvements to ensure that the results of adverse events are presented more coherently and are easier to understand.

The following is revised in the Abstract Section:

Results: The incidence of serious adverse events and grade ≥3 treatment-related adverse events (TRAEs) was 30.9% and 47.3%, respectively. Grade ≥3 immunotherapy-related adverse events occurred in 3 (5.5%) participants. Five (9.1%) participants discontinued treatment due to TRAEs, all of which were grade 1-2.

- *I would also present all results with the time calculated in months and not in weeks.*

Response: Thank you for bringing this matter to our attention. In our previous reporting,

we had presented the exposure duration in weeks. We have now revised all reports to reflect the time calculated in months instead of weeks to ensure consistency.

The following is revised in the Abstract Section:

Results: At the cut-off date of November 30, 2022, fifty-five participants were enrolled; median exposure for KN046 and lenvatinib was 7.1 (0.7-23.4) months and 7.7 (0.3-25.0) months, respectively.

Background

I believe the introduction can be improved by introducing more references to the current literature.

- *Line 63-63: median OS exceeds 22 months in the most recent phase 3 trials (for example, checkmate 9DW), so this should be amended*

Response: We appreciate the Reviewer's insightful comment. After carefully re-evaluating the most recent phase 3 trials, including Checkmate 9DW, we acknowledge that our initial statement regarding the median overall survival (OS) being only 14 months was not accurate.

The following is revised in the Background Section:

Rapid development of interventional therapy and systemic drug therapy has prolonged survival for patients with advanced HCC, but the lack of predictive biomarkers necessitates the need for novel treatment combinations, effective in broader patient populations.

- *If the authors want to mention the mechanism of action of immune checkpoint inhibitors, I would appreciate more detail, as "promoting the destruction of tumour cells" can sound simplistic to the readers of Nature.*

Response: Thank you for your valuable feedback. We have taken your suggestion into

consideration and have revised our introduction on the mechanism of action of immune checkpoint inhibitors (ICIs) to provide a more comprehensive explanation. In particular, we have elaborated on how PD-1/PD-L1 and CTLA-4 inhibitors work synergistically to enhance T cell activation and proliferation during different phases of immune responses [1,2]. We hope this improvement will make the contents more suitable for your readers

The following is revised in the Background section:

Immune checkpoint inhibitors (ICIs) play major roles in immune escape by inhibiting immune responses and promoting immune tolerance toward tumor cells [3]. Among these, cytotoxic T lymphocyte antigen 4 (CTLA-4), programmed death 1 (PD-1) and its ligand (PD-L1) have been identified as promising targets [4]. The combined blockade of ICIs may provide potentially synergistic effects due to the differences in the timing and location of their reactions [2]. For instance, CTLA-4 binds to CD80/86 and prevents the stimulation of T cell proliferation during the priming phase, typically in lymph nodes [1,2]. Conversely, PD-1 pathway regulates activated T cells in peripheral tissues, which impairs proliferation and activation at the later stages of an immune response [1,2].

- *The authors should include updated references to the Phase 3 studies in the field. For example, the Checkmate 9DW tested the double ICI combination of nivo-ipi, or the LEAP 002 (should be added to line 80)*

Response: We appreciate the Reviewer's valuable suggestion. We have incorporated the latest references, including the Checkmate 9DW and LEAP 002 studies, to enhance our background section with a more comprehensive overview of Phase 3 trials in the field. To maintain clarity and avoid repetition, we have also refined the logical flow and structured the introduction more coherently, providing a concise summary of the results.

The following is revised in the Background Section:

Phase III trials demonstrated the efficacy of different combinations of dual

immunotherapy. These included atezolizumab plus bevacizumab (IMbrave150 trial) [5], nivolumab plus ipilimumab (Checkmate 9DW trial) [6], and tremelimumab plus durvalumab (HIMALAYA trial) [7,8], which showed a median OS ranging from 16.4 to 23.7 months and an objective response rate (ORR) ranging from 20.1% to 36% [5-8]. Additionally, LEAP-002 has shown that pembrolizumab combined with vascular endothelial growth factor receptor (VEGFR) inhibitor can achieve ORRs of 26.1%, and the median OS of 21.2 months in patients with HCC [9].

- *Line 84: dual immunotherapy might significantly improve patient survival compared to single agent ICI. This is not true, as the HIMALAYA trial showed. Please reconsider this part.*

Response: We appreciate your suggestion and have removed the sentence in your query. As previously mentioned, we have provided a more comprehensive introduction of phase III trials and ensured an objective presentation of the results.

- *Maybe the authors want to mention the rationale of combining double ICI with Lenvatinib, and maybe mention the reason why they performed the ctDNA analysis (previous data in this setting?)*

Response: Thank you for your insightful comments. The combination of levatinib with ICIs was based on the potential synergistic effects by targeting both angiogenesis and immune escape mechanisms [10]. Additionally, although limited research has been conducted on the combination therapy of dual immune checkpoint inhibitors, one study in gastric or gastroesophageal junction adenocarcinoma has suggested that this combination may mitigate the risk associated with the slow onset of immunotherapy [11]. Regarding the rationale of performing circulating tumor DNA (ctDNA) analysis, we included this methodology as ctDNA may increase the detection rate of certain mutations in HCC patients, which may lead to more personalized treatment strategies [12]. We have updated the Background Section accordingly to reflect these points more

clearly.

The following is revised in the Background Section:

Levatinib, a VEGFR inhibitor, has been shown to provide potential synergistic effects when used in conjunction with ICIs by inhibiting angiogenesis and immunosuppressive responses of the tumor microenvironment [10]. Additionally, study has suggested that this combination may potentially mitigate the risk associated with the slow onset of immunotherapy [11]. The preliminary results of the prospective phase II trial showed encouraging antitumor activity and favorable AE profile of KN046 combined with lenvatinib in unresectable or metastatic HCC [13].

This open-label phase 2 clinical trial aimed to examine the efficacy and safety of KN046 combined with lenvatinib in patients with advanced HCC. Furthermore, previous research have indicated that combining the analysis of circulating tumor DNA (ctDNA) with tumor tissue analysis may increase the detection rate of specific mutations in HCC patients, potentially leading to more personalized treatment options [12]. Therefore, this study also sought to explore the potential biomarkers of prognosis using next-generation sequencing (NGS).

Methods:

- *I believe that the Methods could seem a bit redundant, as most of this information are present in the Study protocol that should be available in the supplementary material.*

Response: We appreciate your feedback and considerations. We have taken steps to streamline and condense the information presented. This includes displaying only the key inclusion and exclusion criteria and removing the dose adjustment guidelines and specific definitions of endpoints. These have been added in the supplementary material. We hope that these changes will make the Methods section more concise and less redundant.

- *A list of the "multi-centers" (line 108, please find an alternative definition) involved in the trial should be provided*

Response: We appreciate your suggestion to provide the information regarding the enrollment centers. As mentioned above, names of centers have been added in the Methods Section.

The following is revised in the Methods Section:

This multi-center open-label phase 2 clinical trial enrolled patients with advanced HCC from September 2020 to October 2021, at Beijing Cancer Hospital and Harbin Medical University Cancer Hospital in China.

- *Line 118: only histological diagnosis is listed. Could the authors explain if radiological diagnosis according to AASLD criteria was allowed? If not, could they confirm that all patients have an available tumour biopsy?*

Response: Thank you for your question. We confirm that this study required all patients to have an available tumor biopsy and undergo pathological diagnosis. This ensured that each patient was accurately diagnosed with hepatocellular carcinoma, thereby guaranteeing the precise administration of medication.

- *Line 121: systematic is a typo, as it should be systemic*

Response: Thank you for your attention to detail. We have corrected this typo.

The following is revised in the Methods:

The key exclusion criteria were 1) fibrolamellar HCC, sarcomatoid HCC, cholangiocarcinoma, mixed type HCC, 2) tumor thrombus invading the main portal vein (Vp4), inferior vena cava, or heart involvement, 3) previously received systemic therapy, immune checkpoint inhibitors (such as anti-PD-1/L1 or CTLA-4 antibodies),

4) received local treatment within 4 weeks before enrollment 5) history of autoimmune diseases, hepatic encephalopathy or liver transplantation.

- *Line 123: fibrous lamina -> fibrolamellar?*

Response: Thank you for bringing this into our attention. Indeed, "fibrous lamina" should have been corrected to as "fibrolamellar." This necessary corrections have been made.

The following is revised in the Methods:

The key exclusion criteria were 1) fibrolamellar HCC, sarcomatoid HCC, cholangiocarcinoma, mixed type HCC, 2) tumor thrombus invading the main portal vein (Vp4), inferior vena cava, or heart involvement, 3) previously received systemic therapy, immune checkpoint inhibitors (such as anti-PD-1/L1 or CTLA-4 antibodies), 4) received local treatment within 4 weeks before enrollment 5) history of autoimmune diseases, hepatic encephalopathy or liver transplantation.

- *Line 146: please use months as measure of time*

Response: Thank you for your valuable suggestion. We have taken your feedback into consideration and updated the measure of time to months for consistency throughout the manuscript.

The following is revised in the Methods:

KN046 was administered until disease progression, intolerable toxicity, or the completion of the planned 27-month treatment. Lenvatinib was administered until disease progression or intolerable toxicity.

- *If I understand correctly, the bispecific Ab was administered up to 2 years, while*

Lenvatinib was allowed even beyond that until disease progression. Could the authors explain the rationale behind this choice?

Response: Thank you for your question. The decision to administer KN046 for up to 2 years and continue lenvatinib until disease progression is based on current research in the field of immunotherapy. Most current studies on immunotherapy involve a treatment duration of two years considering factors including immune-related adverse events. However, if prolonged remission is observed with immunotherapy, it may be feasible to consider maintaining lenvatinib alone to control disease. For instance, in LEAP002 study, participants received lenvatinib daily plus pembrolizumab for up to 35 cycles (approximately 24 months), and lenvatinib was continued until disease progression or unacceptable toxicity [9].

Results

- *Data cutoff: this is a key point to clarify. It seems like there was a data cutoff for most of the analyses (line 261: November 30, 2022) and one for OS (Line 298: March 15, 2024). I believe all the analyses should be presented with a univocal data cutoff.*

Response: We appreciate the insightful feedback and completely agree that for consistency and methodological accuracy, all results should be presented using the same data cut-off. As mentioned above, November 30, 2022 was used as the new unified cut-off for all results reported in Abstract, Results and Figure 3C. This results to an updated of median OS, which is now reflected as 16.4 months (95% CI, 11.20-NR).

The following is revised in the Results section:

Based on imRECIST, the ORR was 45.5% (95% CI, 31.97-59.45), the DCR was 90.9% (95% CI, 80.05-96.98), the median TTR was 2.6 (95% CI, 1.31-2.69) months, and the median DOR was NR (95% CI, 9.33-NR) months (Table 3). A total of 28 (50.9%) PFS events occurred, and the median PFS based on imRECIST was 11.0

(95% CI, 8.21-15.24) months. The median OS was 16.4 (95% CI, 11.20-NR) months, and the 12-month OS rate was 60.0% (95% CI, 45.87-71.55) (Figure 3C).

- *I do not fully understand the reason to present the results according to the dose of Lenvatinib. Lenvatinib is administered at 8 or 12mg according to patients' weight as per summary of product characteristics, but this does not impact efficacy or safety. Many oncological medications are administered at different doses depending on the BSA of the patients, or their weight. I would suggest presenting all the results (including the tables) regardless of Lenvatinib dose, otherwise it might give the false impression that these are two different arms with two different expected outcomes.*

Response: We appreciate your suggestion and agree that the dosage of lenvatinib was not relevant to the study objectives. Consequently, all the tables and figures have been revised to display results regardless of the dosage, and all references to dose cohorts have been removed from the manuscript.

- *The authors should describe in detail the time of previous anticancer therapy (table 1). Inclusion criteria allowed only patients without any previous systemic therapy. Could the authors clarify?*

Response: We thank you for your query. To clarify, our study exclusively enrolled patients who had not received any prior systemic therapy or immunotherapy. Additionally, we excluded patients who had undergone local treatments such as transcatheter chemoembolization, transcatheter embolization, hepatic artery perfusion, radiotherapy, radioembolization, or ablation within 4 weeks before the start of our study. Regarding the medication treatment noted in Table 1, it specifically refers to adjuvant treatment with sorafenib. We have updated this entry to more accurately state "other treatment" and have included an explanatory note under Table 1 to clarify this detail for our readers.

- *In the safety table, I would strongly recommend reporting the overall adverse events, regardless of the attribution of causality to the treatment.*

Response: We appreciate your suggestion and have listed the most common (≥10%) TEAEs in the new Supplementary Table 1.

The following is revised in the Results Section:

The summary of safety analysis is presented in Table 2 and the most common (≥10%) TEAEs are listed in Supplementary Table 1.

- *In the baseline characteristics table, the authors list around 27% of patients with non-viral aetiology. Could they add some further detail, for instance re: MASLD diagnosis? Also, how was cirrhosis diagnosed?*

Response: We appreciate the reviewer's insightful question. Non-viral aetiology of HCC included metabolic dysfunction-associated steatotic liver disease (MASLD), which was diagnosed based on imaging examinations, with the presence of at least one of the three comorbidities: obesity, type 2 diabetes mellitus, or metabolic syndrome in the patient. As for the diagnosis of cirrhosis, it was diagnosed through elastography via ultrasound. To provide clarity and detail, we have added corresponding explanations in the notes section of Table 1.

- Could the authors explain what they mean with “duration of HCC” in table 1?

Response: Thank you for your question about the term “duration of HCC” in Table 1. We have now clarified this by revising it to “time from the initial diagnosis of HCC”.

- *In Figure 1, what do they mean as “investigators’ judgement” as cause of treatment*

discontinuation?

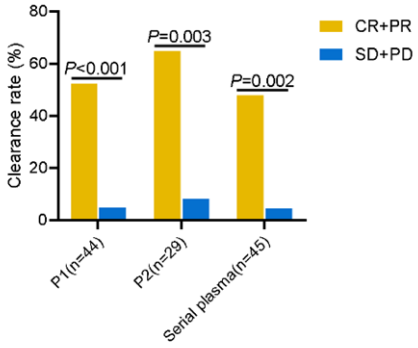
Response: We appreciate your question regarding the term “investigators’ judgement” as a cause of treatment discontinuation in Figure 1. As per the study protocol, if at the conclusion of the study there are still participants undergoing treatment, the investigator will conduct a comprehensive evaluation to determine whether the continued administration of the drug remains beneficial for the subjects. If the investigator believes that continuation of the treatment is not in the best interest of the subject's health or well-being, patients will no longer receive the study drugs.

- *Kaplan meier curves: minor typo “Number of risk” instead of “at risk”*

Response: We thank you for your careful observation and for bringing this typographical error to our attention. The incorrect text “Number of risk” has been corrected to “Number at risk”. The figures have been updated accordingly in the manuscript.

- *In the ctDNA analysis, did the authors identify any difference in ctDNA clearance across responders (CR+PR) vs non-responders?*

Response: We appreciate your insightful query. In response to your question, we have identified significant differences in ctDNA clearance between responders (CR+PR) and non-responders at different time points. The figure attached demonstrates the proportion and difference of ctDNA clearance between CR+PR patients and non-responders. As shown, ctDNA clearance at P1 (the first tumor assessment), P2 (the second tumor assessment), and serial plasma clearance may potentially predict therapeutic efficacy. Among them, P1 shows the most significant difference. Additionally, in the hazard ratio (HR) analysis, we noted that the HR value decreased at P2, indicating a reduction in predictive value for subsequent assessments. Therefore, we have also incorporated a discussion on the value of early dynamic monitoring.



The following is revised in the Discussion Section:

Additionally, the HR analysis has demonstrated significant differences in both PFS and OS based on ctDNA status at P1 (the first tumor assessment). However, the HR value decreased at P2 (the second tumor assessment), indicating a reduction in predictive value for subsequent assessments. In addition, an increasing number of patients had evidence of disease progression at later time points, rendering further ctDNA testing. This highlights the value of early dynamic monitoring.

Discussion

- *I would use caution when describing the safety profile of this combination all across the manuscript. For instance (Line 333) I am not sure it is fully correct to say that the safety profile was manageable, as there is no clear definition of"manageable". 42% of G3-5 trAE is not negligible, and the authors should reinforce this issue.*

Response: We thank Reviewer for the suggestion. This issue was fully revised thtoughout the manuscript. We have thoroughly revised the manuscript to address this concern. All sentences where safety was described as "manageable" or "acceptable" have been removed, and we have reinforced the importance of managing AEs in the Discussion Section. This ensures a more accurate representation of the safety profile of the combination therapy under study.

The following is revised in the Discussion Section:

This open-label phase 2 clinical trial showed that KN046, a bispecific antibody against PD-L1 and CTLA-4, combined with lenvatinib achieved an ORR of over 40% and median PFS of 11.0 months, indicating promising efficacy as first-line treatment for advanced unresectable or metastatic HCC. However, safety concerns have been raised due to the incidence of grade ≥3 TRAEs being 47.3%, underscoring the necessity for appropriate management of AEs.

- *The combination of Lenvatinib-pembrolizumab in the Phase III LEAP 002 trial caused 62% of G3-G4 trAEs. The authors should explain why they think their compound (which also includes an antiCTLA4, notably associated with high rate of immune-related AE) causes a lower rate of AEs, even when combined with Lenvatinib which, alone, was showed to be associated with >50% G3-4 trAE (LEAP 002 control arm Lancet Oncol 2023; REFLECT Lancet 2018). They will need to elaborate on this point further, and consider restructuring the discussion to highlight the toxicity of the combination. Please consider rephrasing line 381 "..were acceptable", as there is no consensus on what "acceptable" should be.*

Response: We appreciate your valuable suggestion. As previously mentioned, we have removed all sentences where safety was described as "manageable" or "acceptable" and have emphasized the importance of managing AEs in the Discussion section. We have also incorporated a discussion on the lower incidence of grade ≥ 3 treatment-related adverse events (TRAEs) with KN046 plus lenvatinib compared to previous studies involving single-agent immunotherapy combined with antiangiogenic therapy. The possible reasons for this difference could be variations in safety profiles among different treatment combinations, as well as differences in the patient populations included in the studies. For instance, our study included 50.9% of participants with extrahepatic disease, while the LEAP-002 and REFLECT trials had higher proportions at 63% and 60.9% [9,14], respectively, which may potentially affect the safety outcomes. Additionally, although not discussed in the manuscript, we believe appropriate AEs

management including close monitoring and timely intervention of AEs in our study, may also contribute to a reduced incidence of grade ≥ 3 TRAEs.

The following is revised in the Discussion Section:

The incidences of grade ≥ 3 TEAEs and grade ≥ 3 TRAEs with KN046 plus lenvatinib were 58.2% and 47.3%, respectively. In previous trials of single-agent immunotherapy plus antiangiogenic therapy, the incidence of grade ≥ 3 TRAEs ranged from 55% to 80.9%, which may potentially lead to early discontinuation of treatment [9,14-16]. The incidence of grade ≥ 3 TRAEs in this study was numerically lower than those previously reported. Despite differences in safety profiles between different treatment combinations, this discrepancy could also stem from variations in the patient populations included in the studies. For example, our study included 50.9% of participants with extrahepatic disease, while the LEAP-002 and REFLECT trials had higher proportions at 63% and 60.9%, respectively [9,14], which may potentially influence the safety results.

- *Line 354: please add the correct reference to the HIMALAYA trial*

Response: We appreciate your attention to detail. The reference of the HIMALAYA trial has been updated accordingly [7]. We acknowledged that there was an updated analysis of HIMALAYA trial regarding long-term OS [8], we still cite the primary analysis as all the data referenced are from the primary analysis [7].

- *Lines 383-385: I am not sure this is in line with current literature. Durva-treme in the HIMALAYA trial were associated with 25.8% of G3-4 trAEs, way less than the combination tested in this manuscript. Please consider restructuring the discussion accordingly.*

Response: We appreciate your suggestion. Upon careful re-evaluation of our manuscript, we acknowledge that the information provided in the Discussion might not precisely convey the differences in AEs due to the different safety profiles of ICIs. To

ensure that our discussion is both accurate and relevant, we have decided to revise the section by removing this sentence.

The following is revised in the Discussion Section:

KN046 is a fully human wild-type IgG1 Fc, which was cleverly designed in tandem with domain antibodies (dAbs) to form a bispecific antibody that simultaneously recognizes PDL1 and CTLA4 and activates the immune response [13,17]. In addition to releasing PDL1-mediated immunosuppression, enhancing CTL immune function, and releasing CTLA4-mediated Treg immunosuppression, bispecific antibodies may cause fewer side effects than two separate PD-1/L1 and CTLA-4 antibodies. Furthermore, a bispecific antibody requires only one infusion, compared with dual immunotherapy, which requires two separate infusions, resulting in longer treatment times and sometimes more premedication [18].

- *I would suggest expanding more the limitations of the study, including that the population enrolled has a univocal geographical origin, and so the conclusions from this study cannot be generalised (for instance, due to the majority of HBV associated HCC).*

Response: We appreciate your valuable suggestion and agree that it is crucial to acknowledge the limitations of our study. To address this concern, we have expanded the limitations section of our manuscript to include a discussion on the potential impact of the univocal geographical origin of our study population.

The following is revised in the Discussion Section:

This trial had several limitations. First, it was a phase II trial with a limited sample size. In addition, there was no control group, preventing the direct evaluation of efficacy compared with standard treatment. Second, the enrolled population consisted of only participants from China, with a relatively high prevalence of viral hepatitis. The impact of HBV on the response to immunotherapy remains a critical factor; hence, the findings

may not be generalizable to other populations with different epidemiological characteristics. Moreover, the follow-up was relatively short. Large-scale, randomized controlled trials with a longer follow-up are needed to confirm the results. Finally, the incorporation of RNA sequencing data could further increase the utility of the biomarker analysis [19]. However, this was not conducted due to the unavailability of additional biological samples. Future studies will consider incorporating RNA sequencing to provide a more comprehensive evaluation.

Reviewer #2 (Remarks to the Author): with expertise in HCC, therapy

1. Lines 94-97: The authors discuss their previous findings in the Introduction (reported in Jiang et al., Adv Sci, 2021: 2102500) as a motivation for this study. However, that study reported that intra-tumoral injection of KN046 in mice can be safely and effectively delivered by the ZIF-8 delivery system. Since the delivery route was different, it is difficult to determine the relevance of those findings for this clinical study.

Response: We are grateful for the Reviewer's insightful feedback and careful consideration of our manuscript. We have revised the Introduction section to provide a more comprehensive overview of the current research and the rationale behind our study. Regarding the referenced study by Jiang et al., we acknowledge that the delivery route differs from our clinical study, which may affect the direct relevance of their findings to our work [20]. However, we would like to clarify that the reference was included primarily to provide background information on the mechanism of action of KN046, rather than to motivate our clinical study design. The primary motivation for our study stems from existing clinical trials and the potential synergistic effects of combination therapy. To avoid any misunderstandings, we revised the wording accordingly.

The following is revised in the Background Section:

The KN046 is a bispecific antibody targeting the PD-1/PD-L1 and CTLA-4/B7 immune checkpoint pathways [20]. Preclinical study showed that KN046 has a higher affinity for

PD-L1 and reduced side effects due to its specific structural modification, making it a potential candidate for further development as an immunotherapy agent [20]. Recent clinical trials showed that KN046 is well tolerated as a monotherapy in patients with advanced solid tumors [21] or in combination with chemotherapy in patients with triple-negative breast cancer [22]. Levatinib, a VEGFR inhibitor, has been shown to provide potential synergistic effects when used in conjunction with ICIs by inhibiting angiogenesis and immunosuppressive responses of the tumor microenvironment [10]. Additionally, study has suggested that this combination may potentially mitigate the risk associated with the slow onset of immunotherapy [11]. The preliminary results of the prospective phase II trial showed encouraging antitumor activity and favorable AE profile of KN046 combined with lenvatinib in unresectable or metastatic HCC [13].

2. On page 8, Lines 194-195, the description of the time points used for ctDNA collection is unclear. Please clarify when p3 is in the methods. Are p2 and p3 the same time point?

Response: We appreciate your feedback. In our study, plasma cfDNA samples were collected at various stages: before treatment (P0, n=49), after the first tumor assessment (P1, n=48), after the second tumor assessment (P2, n=30), and at the time of disease progression (PD) (P4, n=7). Additionally, plasma samples from six patients were collected when the tumor evaluation changed from stable disease (SD) to partial response (PR), previously denoted as P3. This P3 time point signifies the transition in tumor evaluation and was only available for six patients, with two instances where P3 coincided with P2. To enhance the clarity of our conclusions, we decided to omit the analysis results pertaining to the P3 point in this revised version. The manuscript has been revised accordingly, which includes updates to Figure 4C, 4E, S3B, S3C, and S3E, accompanied by corresponding adjustments in the text throughout the manuscript.

The following is revised in the Methods Section:

Baseline tissue samples were available in 44 patients. Plasma cfDNA samples were

collected before treatment (P0, n=49), before the third cycle of treatment (P1, n=48), before the fifth cycle of treatment (P2, n=30), and at the time of PD (disease progression) (P3, n=7). Serial plasma was defined as plasma from patients with any of the points P1/P2, which was available in 49 patients. Serial plasma positive was defined as at least one positive result in either P1 or P2. Due to the limited sample size of P3, it was excluded from the data analysis in this study.

3. On page 22, the description of the Clinical Study Protocol KN046-IST-05: Why were dose adjustments of KN046 not allowed? What was the management for the patients with AE grade>3 during the period of treatment interruption?

Response: We appreciate your valuable feedback. In response to your question, dose adjustments for KN046 were not allowed based on the previous experience that for ICI drugs. Previous phase I trial in patients with advanced solid tumors has shown that there was no obvious relevance between KN046 dosage and the incidence of AEs [21]. Given the weak relationship between exposure level and response, reducing the dosage in participants who experience severe high-grade AEs may still lead to a recurrence of these high-grade AEs. Additionally, for anti-PD-1/PD-L1 and anti-CTLA-4 medications which have been approved, their instructions do not usually recommend dose reduction as a method of managing AEs. Instead, they suggest measures such as temporarily stopping or permanently discontinuing the drug depending on the severity level of the AE. We have also included the management for the patients with grade ≥3 AE during the period of treatment interruption for your reference.

In case of grade ≥3 AE occurring during the period of treatment interruption, the following may be considered based on investigator's discretion:

1. Delay immunotherapy.
2. Symptomatic management: patients received supportive care tailored to the specific adverse event they experienced.
3. Use of steroids or immunosuppressants.
4. Close monitoring: patients were closely monitored with regular clinical assessments

and laboratory tests to track the progression of the adverse event and evaluate their recovery.

5. Gradual reintroduction of treatment: once the adverse event was resolved to grade ≤1 or returned to baseline levels, the treatment was gradually reintroduced at a reduced dose.

6. Permanent discontinuation: In cases where the adverse event did not resolve or recurred upon reintroduction of treatment, the therapy was permanently discontinued, and the patient was managed with alternative treatments.

4. Figure 4: The analysis of RNA sequencing data for the immune classification of HCC (Kurebayashi et al., Hepatology. 2022; 75(5): 1139-1153) would be useful to increase the utility of the biomarker.

Response: We thank you for the valuable suggestion regarding the use of RNA sequencing data for the immune classification of HCC. This approach could indeed enhance the utility of our biomarker. Unfortunately, we do not have access to additional samples to perform this analysis at this time. We value your input and will consider it in future research. This limitation was also added in the Discussion Section. Thank you once again for your insightful recommendation.

The following is revised in the Discussion Section:

This trial had several limitations. First, it was a phase II trial with a limited sample size. In addition, there was no control group, preventing the direct evaluation of efficacy compared with standard treatment. Second, the enrolled population consisted of only participants from China, with a relatively high prevalence of viral hepatitis. The impact of HBV on the response to immunotherapy remains a critical factor; hence, the findings may not be generalizable to other populations with different epidemiological characteristics. Moreover, the follow-up was relatively short. Large-scale, randomized controlled trials with a longer follow-up are needed to confirm the results. Finally, the incorporation of RNA sequencing data could further increase the utility of the

biomarker analysis [19]. However, this was not conducted due to the unavailability of additional biological samples. Future studies will consider to incorporating RNA sequencing to provide a more comprehensive evaluation.

5. Line 362-365, 387-390: The previous papers have indicated KN046 has promising antitumor efficacy. Please discuss the mechanisms of potential synergy (and toxicity) between lenvatinib and KN046.

Response: Thank you for your feedback and suggestion. We have revised the discussion section to better address the mechanisms of potential synergy between lenvatinib and KN046, as well as highlighted the potential additive toxicity in the Discussion Section.

The following is revised in the Discussion Section:

Previous research indicated that combining anti-VEGF therapy with ICIs could provide a synergistic effect, due to the immunomodulatory role of VEGF-A. This factor stimulates activity of immune suppressor cells, including tumor-associated macrophages, Treg cells, and myeloid-derived suppressor cells [23]. As a result, combining antiangiogenic therapy with ICIs may potentiate the counteraction against tumor immunity suppression. The results of this study, consistent with previous findings, suggest that the synergistic combination may lead to higher, durable response and improve survival in patients with advanced HCC. Moreover, the duration of treatment in this study was longer than in previous studies [24], and the proportion of patients who achieved durable clinical response was also higher than when using two separate antibodies against de PD-1/L1 and CTLA-4/B7 axes [24]. The ORR in the present study (46%) was higher than 13%-30% in previous clinical trials [5,9,15,16]. These results highlight the good prospects of bispecific antibodies for immunotherapy in HCC.

KN046 is a fully human wild-type IgG1 Fc, which was cleverly designed in tandem with domain antibodies (dAbs) to form a bispecific antibody that simultaneously recognizes PDL1 and CTLA4 and activates the immune response [13,17]. In addition to

releasing PDL1-mediated immunosuppression, enhancing CTL immune function, and releasing CTLA4-mediated Treg immunosuppression, bispecific antibodies may cause fewer side effects than two separate PD-1/L1 and CTLA-4 antibodies. Furthermore, a bispecific antibody requires only one infusion, compared with dual immunotherapy, which requires two separate infusions, resulting in longer treatment times and sometimes more premedication [18]. However, it is worth noting that the inclusion of an anti-angiogenic agent may result in a higher incidence of adverse events, which necessitates close monitoring for potential side effects in clinical settings [25].

6. Based on the ctDNA biomarker analysis results at the pre-treatment time point (P0), can the authors discuss more clearly which genetic variations detected by this non-invasive method might predict a patient's non-responsiveness? This could help identify patients who are more likely to respond to the treatment before its administration (as a predictive biomarker). This will be more useful than a prognostic biomarker (which defines the outcomes irrespective of treatment). Moreover, a randomized study will be required to determine whether the dynamic biomarker is prognostic or predictive.

Response: We appreciate the reviewer's insightful suggestion. To address this point, we have added a new figure, designated as Supplementary Figure 2, while the original Supplementary Figure 2 has been renamed to Supplementary Figure 3. Supplementary Figure 2A shows the relationship between mutant genes in P0 plasma and ORR, with significant differences observed with TP53, TERT, and CTNNB1. The relationship between mutant genes in P0 plasma and prognosis is shown in Supplementary Figure 2B-I. Amplification of CCND1 (B, C) and mutation of TP53 (F, G) was significantly associated with worse PFS and OS; mutation of FGF19 (E) and TERT (I) was significantly associated with worse OS, but not significantly related to PFS (D, H). The text in the manuscript and the supplemental materials have also been correspondingly added and revised.

The following is revised in the Results Section:

Unlike the findings in baseline tumor tissues, TP53, TERT and CTNNB1 mutations were significantly more frequent in non-responders (patients with SD+PD) than in responders (patients with CR+PR) concerning baseline plasma (P0) (Supplementary Figure 2A). Patients harboring CCND1 CNV or TP53 mutation showed unfavorable PFS and OS (Supplementary Figure 2B, C, F, G); while those harboring FGF19 CNV or TERT mutation showed unfavorable OS (Supplementary Figure 2E, I), but not PFS (Supplementary Figure 2D, H).

7. The manuscript would benefit from editing for English.

Response: We appreciate the reviewer's suggestion for further editing. In response, we have conducted a thorough review of the manuscript to refine the language and correct any grammatical or spelling errors. We believe these efforts will significantly enhance the overall readability and impact of our work.

Reviewer #3 (Remarks to the Author): with expertise in HCC, therapy

1. In this study, "TRAEs occurred in 100% of the participants, of which grade ≥3 occurred in 26 (47.3%)." "Thirteen (23.6%) participants experienced irAEs." In the table 2, we can conclude that 54 (98.2%) cases suffered from TRAE and 15 (27.3%) cases Grade ≥3. Regarding the drug KN046, it is noteworthy that the incidence of adverse reactions is indeed very low, which is quite contrary to our previous impressions of CTLA-4 antibodies or bispecific antibodies. This raises the question of whether the researchers employed any specific strategies for controlling or preventing adverse reactions?

Response: Thank you for your feedback. Regarding the low incidence of adverse reactions with KN046 plus lenvatinib, we acknowledge that this is different from previous research, especilally when compared to previous studies involving single-agent immunotherapy combined with antiangiogenic therapy. To address this concern, we have added a discussion about possible reasons for this discrepancy in our

manuscript. The possible reasons for this difference could be variations in safety profiles among different treatment combinations, as well as differences in the patient populations included in the studies. For instance, our study included 50.9% of participants with extrahepatic disease, while the LEAP-002 and REFLECT trials had higher proportions at 63% and 60.9% [9,14], respectively, which may potentially affect the safety outcomes. Additionally, although not discussed in the manuscript, we believe appropriate AEs management including close monitoring and timely intervention of AEs in our study, may also contribute to a reduced incidence of grade ≥ 3 TRAEs.

The following is revised in the Discussion Section:

The incidences of grade ≥ 3 TEAEs and grade ≥ 3 TRAEs with KN046 plus lenvatinib were 58.2% and 47.3%, respectively. In previous trials of single-agent immunotherapy plus antiangiogenic therapy, the incidence of grade ≥ 3 TRAEs ranged from 55% to 80.9%, which may potentially lead to early discontinuation of treatment [9,14-16]. The incidence of grade ≥ 3 TRAEs in this study was numerically lower than those previously reported. Despite differences in safety profiles between different treatment combinations, this discrepancy could also stem from variations in the patient populations included in the studies. For example, our study included 50.9% of participants with extrahepatic disease, while the LEAP-002 and REFLECT trials had higher proportions at 63% and 60.9%, respectively [9,14], which may potentially influence the safety results.

2. We are concerned that in this study, a significant number of patients received 12mg Lenvatinib qd, so what are the similarities and differences in the incidence of Lenvatinib-related adverse reactions among patients in different dose groups? What are the differences in effectiveness between the two groups?

Response: We appreciate your insightful suggestion. In response to Review#1, we acknowledge that the dosage of lenvatinib was not directly relevant to the objectives of our study. Consequently, all the tables and figures have been revised to display results

regardless of the dosage, and all references to dose cohorts have been removed from the manuscript.

2. In this study, 44 patients retained baseline tissue samples. Plasma was available for 50 patients at some point in time. We understand that this is a very large amount of work, showcasing the dedication and thoroughness of the researchers. Specifically, we seek clarity on the protocols employed for acquiring these tissue specimens, as their acquisition is paramount to the integrity and validity of the research findings. Additionally, we are concerned about any potential adverse reactions or complications associated with these procedures, as we prioritize patient safety above all else.

Response: We thank you for your question and appreciate your considerations for the patients. Before undergoing treatment, patients must obtain tissue samples through ultrasound-guided liver tumor biopsy and undergo histopathological examination to confirm the diagnosis of hepatocellular carcinoma before proceeding with treatment. Prior to the biopsy, patients were required to sign an informed consent form for the procedure, ensuring their right to be informed while also clearly discussing with them all the risks associated with the biopsy, such as bleeding, tumor spread, or the inability to obtain conclusive active tumor tissue to confirm hepatocellular carcinoma. To minimize the risks and ensure the safety of the biopsy procedure we consistently engaged experienced hepatic specialists from the ultrasonography department (with over 20 years of experience). Additionally, the chosen biopsy needles were equipped with double-sided sheaths, allowing for the protection of surrounding tissue by the outer sheath when the inner sheath containing the tumor tissue is removed, thereby preventing any risk of tumor cell dissemination into healthy tissues. For patients whose biopsies fail to confirm hepatocellular carcinoma or do not yield malignant liver tumor cells, this actually serves as a protective measure, avoiding indiscriminate medication use.

4. We have noted that baseline tissue specimens were retained in this study, which

prompts us to inquire about the detection strategy for ctDNA: is it tumor-informed or tumor-agnostic? This question arises particularly because we also observe that the number of patients from whom blood samples were collected exceeds the number of tissue specimens.

Response: We thank you for your question. In response to your inquiry, the method was tumor-informed, but the threshold for detection was adjusted based on the results of tumor tissue testing. A fixed NGS large panel was used for testing, and both tissue mutations and plasma-specific mutations were taken into account. Detection threshold requirements of plasma ctDNA for the mutations detected in tissues wer: 1) for hotspot SNV or indel: alter reads≥3, depth≥100X; 2) for non-hotspot SNV or indel: alter reads≥6, depth≥100X. Detection threshold requirements of plasma ctDNA for the mutations undetected in tissues: for all SNV or indel, VAF≥1%, or alter reads≥6, depth≥100X. Plasma ctDNA is considered positive if any true mutation is detected in the sample.

5. In this study, 54.5% of the patients did not achieve an objective response. May we inquire whether these patients received any corresponding subsequent line of treatment? Furthermore, do the researchers believe that these subsequent treatments had any impact on the patients' survival?

Response: We thank Reviewer for the question. In this study, patients who did not achieve an objective response were not necessarily required to undergo subsequent-line therapy. As long as the patient's tumor remained stable or continued to show remission, they continued with the treatment regimen of this study. Only when a confirmed PD was observed during tumor evaluation did patients receive second-line therapy. The choice of second-line treatment strictly followed clinical practices and guideline recommendations, including atezolizumab plus bevacizumab, regorafenib, and TACE interventional treatment in some patients. We believed that active second-line therapy offers patients better survival benefits.

6. Have the researchers continued to follow up on the long-term survival data of this cohort? The potential long-term survival benefits of this cohort could be attractive. Of course, the case number in a Phase II study is limited, and its conclusions need further validation. Hence, we eagerly anticipate the corresponding Phase III study. Do the researchers have any plans for such a study?

Response: We are grateful for your inquiry. While we currently do not have plans for follow-up, we do have plans for further studies. However, we cannot provide specific details regarding any upcoming studies at this time.

Reviewer #4 (Remarks to the Author): with expertise in clinical trial study design, biostatistics

1. Abstract Line 49: Could the author confirm what circulating tumour DNA status at baseline are they referring to? Based on Figure S2C, P0 was not associated with either PFS or OS.

Response: We appreciate the Reviewer's insightful comments and suggestions. To address the concern, we would like to clarify that when P0 is combined with P1, patients who are ctDNA-negative at both time points exhibit improved PFS and OS. This observation may be of interest for future research. However, it is important to note that the prognostic value of the single time point P0 alone was not associated with patient outcomes. To avoid any potential confusion, we have removed the reference to the baseline (P0) in the Abstract.

The following is revised in the Abstract Section:

Results: At the cut-off date of November 30, 2022, fifty-five participants were enrolled; median exposure for KN046 and lenvatinib was 7.1 (0.7-23.4) months and 7.7 (0.3-25.0) months, respectively. No DLT was observed in the safety run-in period. The incidence of serious adverse events and grade ≥3 treatment-related adverse events (TRAEs) was 30.9% and 47.3%, respectively. Grade ≥3 immunotherapy-related

adverse events occurred in 3 (5.5%) participants. Five (9.1%) participants discontinued treatment due to TRAEs, all of which were grade 1-2. The ORR was 45.5% (95% CI, 31.97-59.45). The median progression-free survival was 11.0 (95% CI, 8.21-15.24) months. The median overall survival (OS) was 16.4 (95% CI, 11.20-not reached) months, and 12-month OS rate was 60.0% (95% CI, 45.87-71.55). Circulating tumor DNA status before the third cycle of treatment was associated with prognosis.

2. Endpoints Line173-175, could the authors include information on how censorings were handled for PFS and OS; when are patients censored for these endpoints.

Response: We appreciate your inquiry regarding the handling of censorings for PFS and OS. We have attached two tables detailing the censorings methods for your reference.

Description of Event and Censor	**PFS Status**	**Date of Event or Censor**
Record of disease progression	Event	Date of disease progression
Death before the record of the earliest disease progression	Event	Date of death
No baseline assessment	Censor	Date of the first administration of treatment
No post-baseline assessment	Censor	Date of the first administration of treatment
Death or disease progression after at least	Censor	Date of the most recent available assessment

two consecutive missing assessments		before the missing assessments
False positive diagnosis of disease progression	Censor	Date of the most recent available assessment
Initiation of new anti-cancer treatment	Censor	Date of the most recent available assessment before the initiation of new anti-cancer treatment
No event occurred during the study	Censor	Date of the most recent available assessment
Withdrawal without evidence of event	Censor	Date of the most recent available assessment
Loss to follow-up	Censor	Date of the most recent available assessment

Description of Event and Censor	**OS Status**	**Date of Event or Censor**
Death	Event	Date of death
No death event occurred during the study	Censor	Date of the last known survival date before data cut-off
Loss to follow-up without evidence of death	Censor	Date of the last confirmed survival date
Withdrawal	Censor	Date of the last confirmed survival date
No death event occurred before the study ends	Censor	Date of the last confirmed survival date

3. Discussion Line 358-360, the median PFS was 11.0 (95% CI: 8.21-15.24) months, it would not be correct to state that the median PFS exceeded 11.0 months.

Response: We appreciate your suggestion and have made the necessary correction.

The following is revised in the Discussion Section:

The present study innovatively used a bispecific antibody plus lenvatinib; the ORR reached about 50%, and the median PFS exceeded reached 11 months.

4. Please include in the statistical analysis section what method was used to compute the median follow up.

Response: We appreciate the reviewer's insightful comment regarding the statistical analysis section. In our study, the median follow-up time was calculated using the reverse Kaplan-Meier method. Participants with death events were censored, while those who survived were considered as events. This method has been incorporated to the Statistical Analysis Section.

The following is revised in the Methods Section:

The medians and 95% CIs of DOR, PFS, and OS were estimated using the Kaplan-Meier method. The Kaplan-Meier survival curve was utilized to analyze PFS and OS among different patient groups, with statistical differences assessed using the log-rank test. Reverse Kaplan-Meier method was used for calculating the median follow-up time. For categorical variables, Fisher’s exact test was used. All statistical analyzes were performed using SAS software version 9.4 (SAS Institute Inc., Cary, NC, USA) or R software version 4.1.0 (R Foundation for Statistical Computing, Vienna, Austria), and a two-sided $P < 0.05$ was considered to be statistically significant.

5. Please include in the statistical analysis section what method was used to compute the hazard ratios reported.

Response: We appreciate your suggestion. In our previous analysis, we utilized a Cox

proportional hazards regression model to compute the hazard ratios reported in the manuscript. However, following the recommendations from previous reviewers, we have removed the results pertaining to different dosage cohorts. As a result, the HR value was no longer applicable in this context. Consequently, we did not include this information in the statistical analysis section of the revised manuscript.

6. Table 1. For Duration of HCC and total diameter of target lesions (RECIST and mRECIST), could the authors please clarify what are presented? Do they represent mean with standard deviation, median with interquartile range or range or others?

Response: We appreciate your attention to details. In Table 1, the “Duration of HCC” (changed to “Time from initial diagnosis of HCC”) is presented as median (IQR), while total diameter of target lesions is presented as mean±SD. Corresponding clarifications were added to the Table and footnotes.

Reviewer #5 (Remarks to the Author):

I co-reviewed this manuscript with one of the reviewers who provided the listed reports. This is part of the Nature Communications initiative to facilitate training in peer review and to provide appropriate recognition for Early Career Researchers who co-review manuscripts.

Response: We are grateful for the time and effort you invested in co-reviewing our manuscript. Thank you.

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Response to Reviewers' comments

Reviewer #1 (Remarks to the Author):

I would like to thank the authors for addressing the reviewers' comments. By doing so, I believe their manuscript has remarkably improved.

I would like to reinforce the importance of describing the HCC aetiology with more granularity. In any manuscript about HCC, aetiology is reported as being related to HBV, HCV, or non-viral. The non-viral category encompasses not only metabolic- related HCC (such as MASLD), but also patients with HCC due to alcohol consumption or due to an unknown cause. I believe the authors should clarify whether the 27% patients with "other aetiology" (Table 1) were all diagnosed with MASLD. I would recommend breaking down the "Others" aetiology (Table 1) into metabolic liver disease (MASLD), alcohol, and unknown, in line with the reporting practice in the current literature.

Response: We are grateful for your insightful suggestions. In response, we have revised Table 1 to provide a clearer breakdown of the “other aetiology” category in the baseline characteristics, specifically identifying cases of metabolic liver disease (MASLD), alcohol-associated liver disease, and unknown causes.

Reviewer #2 (Remarks to the Author):

The authors have revised the manuscript and have addressed appropriately all my concerns.

Response: Thank you for acknowledging our revisions. We appreciate your constructive feedback that has enhanced our manuscript.

Reviewer #3 (Remarks to the Author):

The authors have provided satisfactory responses to the questions raised in our review comments and supplemented the paper with corresponding detailed information. We acknowledge the efforts made by the author team. As before, we believe that this is a meaningful clinical study with research results that are highly relevant to clinical practice. There are still some parts of the article that require careful polishing by English professionals. In our opinion, the article should be accepted for publication.

Response: We sincerely thank you for your positive remarks and for your suggestion regarding language refinement. The manuscript has been polished again by a native speaker to improve clarity and flow.

Reviewer #4 (Remarks to the Author):

The authors have satisfactorily addressed my comments, except for comments 2 and 5.

With regards to comment #2, could the author confirm if the censoring information for PFS and OS are included in the manuscript or the accompanying protocol/statistical analysis plan/supplementary materials?

Response: Thank you for your thoughtful comments. We have added the censoring information for PFS and OS in the Supplementary Materials (Page 6-7).

With regards to comment #5, the HRs reported in the previous manuscript were not related to the drug dosing, hence, the removal of dosage cohorts would not render it unnecessary to define the method used to estimate the HRs reported in this manuscript. HRs were reported in Figure 4(D and E) and Supplementary Figure 3 (C-E) of the revised manuscript.

Response: Thank you for your insightful comment. We have revised the Methods section to specify that hazard ratios (HR) with 95% confidence intervals (CI) were estimated using Cox proportional hazards regression analysis.

The following is revised in the Methods Section:

All statistical analyses were performed using SAS software version 9.4 (SAS Institute Inc., Cary, NC, USA) or R software version 4.1.0 (R Foundation for Statistical Computing, Vienna, Austria), hazard ratios (HR) with 95% confidence intervals (CI) were estimated by the Cox proportional hazards regression analysis, and a two-sided P <0.05 was considered to be statistically significant.

Given the change in the data used for the overall survival analysis, I have an additional comment. Please note that the median OS was estimated as 16.4 months, while the upper limit of the 95% CI for the median OS is "not estimable" instead of "not reached". This is presented correctly in Figure 3C but not in the main manuscript text.

Response: Thank you for the suggestion. We have corrected the text regarding the median OS, which is now consistently described as 16.4 months with the upper limit of the 95% CI noted as "not estimable".

The following is revised in the Abstract Section:

The median overall survival (OS) was 16.4 (95% CI, 11.20-not estimable) months, and 12-month OS rate was 60.0% (95% CI, 45.87-71.55).

The following is revised in the Results Section:

The median OS was 16.4 (95% CI, 11.20-not estimable) months, and the 12-month OS rate was 60.0% (95% CI, 45.87-71.55) (Figure 3C).

Reviewer #5 (Remarks to the Author):

I co-reviewed this manuscript with one of the reviewers who provided the listed reports. This is part of the Nature Communications initiative to facilitate training in peer review and to provide appropriate recognition for Early Career Researchers who co-review manuscripts.

Response: We sincerely thank the reviewer for your valuable time.

Once again, thank you for your consideration and we hope that you will find the revised paper suitable for publication. Please let us know if there are any further requirements or changes needed to finalize the publication process.