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# Hepatocellular carcinoma: From personalized medicine to practical guidelines

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**Published in**
Frontiers in Pharmacology
Frontiers in Immunology

ISSN 1664-8714
ISBN 978-2-8325-3953-8
DOI 10.3389/978-2-8325-3953-8

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# Hepatocellular carcinoma: From personalized medicine to practical guidelines

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**Citation**
El-Nakeep, S., Shwaky, A. S., Kasi, A., Rachel, W., Aung, H., eds. (2023). *Hepatocellular carcinoma: From personalized medicine to practical guidelines*. Lausanne: Frontiers Media SA. doi: 10.3389/978-2-8325-3953-8

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frontiers | Frontiers in Pharmacology

TYPE Editorial
PUBLISHED 02 November 2023
DOI 10.3389/fphar.2023.1301202



OPEN ACCESS

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RECEIVED 24 September 2023
ACCEPTED 25 October 2023
PUBLISHED 02 November 2023

CITATION
El-Nakeep S and Kasi A (2023), Editorial: Hepatocellular carcinoma: from personalized medicine to practical guidelines.
*Front. Pharmacol.* 14:1301202.
doi: 10.3389/fphar.2023.1301202



# Editorial: Hepatocellular carcinoma: from personalized medicine to practical guidelines

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KEYWORDS

**HCC, personalized medicine, molecular pathway, biomarkers, current guidelines**

Editorial on the Research Topic
Hepatocellular carcinoma: from personalized medicine to practical guidelines

## Introduction

Hepatocellular carcinoma (HCC) is the most common primary liver malignancy, with a survival rate of 18%. The recent Barcelona 2022 guidelines updated the liver transplantation category, including a downstaging group by transarterial chemoembolization (TACE) and *first-line* combination therapy with atezolizumab and bevacizumab for advanced HCC (aHCC) (Tsilimigras et al., 2022). These small steps towards increasing the options for patients who are considered advanced pave the way for improving survival. Molecular and immunotherapeutic drugs are available only for aHCC (Reig et al., 2022) with low survival benefits (Sun H. et al.). Moreover, there is no preferred regimen for the first- or second-line treatment of aHCC, despite multiple approved Food and Drug Administration (FDA) molecules. A policy review suggested a multiparametric therapeutic hierarchy, from a surgical approach to systemic therapy, offered according to the survival benefit to the individual patient, was proposed recently (Vitale et al., 2023). Personalized medicine using specific molecular pathway modifications is limited to vascular endothelial growth factor inhibitors (VEGFI), tyrosine kinase inhibitors (TKI), and immune checkpoint inhibitors (ICI). A meta-analysis using ICI combined with VEGFI as first-line therapy for aHCC, on 3168 patients, showed that this combination was safe and tolerable, with a pooled median OS of 14.7 months (Gao et al., 2023). The combination of local and systemic approaches is promising. A cohort showed a median OS of 21.8 months with the combination of sorafenib and local hepatic artery infusion of chemotherapy after TACE (Liu et al., 2020).

Limitations of personalized medicine are illustrated in Figure 1. First, prior availability of histopathological studies is lacking, as tissue biopsy is not a common procedure in HCC cases, only retrospective specimens are available from resection.

Second, biomarker expression is mostly heterogeneous, with multiple affected molecular pathways and genetic mutations and no biomarker-driven therapy pathway, such as in lung and colon cancers, is determined. Moreover, patients with low or negatively expressed biomarkers may respond to targeted therapy similar to those with positively expressed biomarkers (Chan et al., 2022).

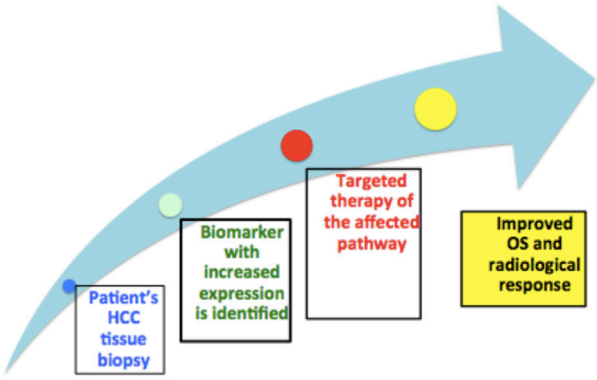


FIGURE 1
Proposed personalized approach and points of limitations.

Additionally, cirrhosis surrounding the cancer with different sets of mutated molecular pathways caused by chronic inflammation needs to be treated differently from cancer (Chan et al., 2022).

Third, resistance to targeted therapy occurs owing to intratumoral heterogeneity, as each area has different molecular pathway mutation; resulting in tumor "flare," and worsening the prognosis (Chan et al., 2022).

Finally, "financial toxicity" (Gyawali, 2017) is of concern given the ethical issue of comparing new drugs to placebo, and not the standard treatment, or the clinical preference of drugs with a meager increase in survival by 1–3 months over that of the standard therapy, with a higher cost.

Hope lies in personalized medicine. First, is the ability to decide beforehand which molecular pathway modification is most suitable according to genetic or molecular testing. Only one FDA-approved test using next-generation sequencing for tumor profiling includes 468 genes (MSA-IMPACT) (Jibiki et al., 2021). The test detects drug resistance using sorafenib and immunotherapy (Dominguez and Wang, 2020). Only one biomarker-targeted therapy, ramucirumab, is FDA-approved after the REACH-2 trial, which increases the OS for patients with aHCC who failed sorafenib with an AFP ≥ 400 ng/mL. The placebo group had an OS of 7.3 months vs. 8.5 months in the active group (Zhu et al., 2019). However, its mechanism of action remains unclear.

Second, drug docking was used to identify novel molecular pathway drugs. Artificial intelligence could be crucial in future testing of molecular models, decreasing the time required for preclinical validation.

Our Research Topic includes five reviews, two cohort studies, a bibliometric and case study. Guan et al. present an excellent review which discusses second-line switching in aHCC using TKI, VEGF, and/or ICIs after failure of the first combination treatment. Single switching is shown to be better than double drug switching in terms of OS and disease progression. Moreover, lenvatinib retention improved survival after switching to a single drug.

The cohort study by Lei et al. comparing TKI monotherapy to TKI combined with PD-1 as a secondary treatment after sorafenib failure, resulted in improved median OS of 21.9 months in the combination group vs. 16.6 months in the monotherapy group.

Another continuous cohort study by Li et al. explored the use of lenvatinib in post-viral HCC; a higher efficacy was observed on HBV-infected than on HCV-infected patients.

An informative review article by Sun L. et al. discusses immune-related adverse events (irAEs) of various immunotherapies. The most common irAEs were cutaneous, gastrointestinal, and hepatic. PD-1 and PD-L1 inhibitors caused dose-independent irAEs, whereas CTLA-4 inhibitors caused dose-dependent irAEs. This review highlights the need for conducting further research on the importance of irAEs as a limiting factor in the treatment of patients, resulting in withdrawal or decrease in the dose of the drug.

The valuable bibliometric study by Wang et al. provides a scientometric analysis of the research published on lenvatinib in HCC and shows an annual growth of 102.5% in this Research Topic.

The review by Sun H. et al. is of practical use and discusses the available targeted therapies in medical practice. First- and second-line therapies approved by the FDA, including TKI, VEGFI, and PD-1 inhibitors used as monotherapy provide a 14–16 months survival rate with serious dose-related side effects and drug resistance. Combination therapy between different categories showed a safety profile similar to that of monotherapy, with some improvement in survival rates by 4–6 months. However, the optimal combination regimen remains undetermined. Bioengineering in the form of patient-derived organoids, patient-derived xenografts, and 3D printing could allow for further personalized approaches.

The detailed review article by Xiao et al. focuses on the role of hypoxia-inducible factors in the recurrence of HCC after ablation by inducing the VEGF pathway.

The minireview by Jiang et al. discusses immunotherapy in post-liver transplantation recurrence. The authors recommended caution in using immunotherapy in this category, as it may result in graft rejection.

Finally, Park et al. present the promising case of a 57-year-old male patient with aHCC treated with a combination of immunotherapy and the anticancer herbal extract Gun-Chil-Jung, who had an OS of 20.3 months.

Overall, our Research Topic covers a snippet of advances in personalized medicine in the literature and highlights the need for further drug research, weighing the balance between survival benefits and hazardous adverse events.

## Author contributions

SE-N: Writing–original draft, Writing–review and editing. AK: Writing–review and editing.

## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

## Publisher's note



## References

Chan, S. L., Wong, N., Lam, W. K. J., and Kuang, M. (2022). Personalized treatment for hepatocellular carcinoma: current status and future perspectives. *J. Gastroenterol. Hepatol.* 37 (7), 1197–1206. doi:10.1111/jgh.15889

Dominguez, D. A., and Wang, X. W. (2020). Impact of next-generation sequencing on outcomes in hepatocellular carcinoma: how precise are we really? *J. Hepatocell. Carcinoma* 7, 33–37. doi:10.2147/jhc.s217948

Gao, X., Zhao, R., Ma, H., and Zuo, S. (2023). Efficacy and safety of atezolizumab plus bevacizumab treatment for advanced hepatocellular carcinoma in the real world: a single-arm meta-analysis. *BMC Cancer* 23 (1), 635. doi:10.1186/s12885-023-11112-w

Gyawali, B. (2017). Low-value practices in oncology contributing to financial toxicity. *Ecancermedicalscience* 11, 727. doi:10.3332/ecancer.2017.727

Jibiki, T., Nishimura, H., Sengoku, S., and Kodama, K. (2021). Regulations, open data and healthcare innovation: a case of MSK-IMPACT and its implications for better cancer care. *Cancers (Basel)* 13 (14), 3448. doi:10.3390/cancers13143448

Liu, B. J., Gao, S., Zhu, X., Guo, J. H., Zhang, X., Chen, H., et al. (2020). Sorafenib combined with embolization plus hepatic arterial infusion chemotherapy for inoperable hepatocellular carcinoma. *World J. Gastrointest. Oncol.* 12 (6), 663–676. doi:10.4251/wjgo.v12.i6.663

Reig, M., Forner, A., Rimola, J., Ferrer-Fàbrega, J., Burrel, M., Garcia-Criado, Á., et al. (2022). BCLC strategy for prognosis prediction and treatment recommendation: the 2022 update. *J. Hepatology* 76 (3), 681–693. doi:10.1016/j.jhep.2021.11.018

Tsilimigras, D. I., Aziz, H., and Pawlik, T. M. (2022). Critical analysis of the updated Barcelona clinic liver cancer (BCLC) group guidelines. *Ann. Surg. Oncol.* 29 (12), 7231–7234. doi:10.1245/s10434-022-12242-4

Vitale, A., Cabibbo, G., Iavarone, M., Viganò, L., Pinato, D. J., Ponziani, F. R., et al. (2023). Personalised management of patients with hepatocellular carcinoma: a multiparametric therapeutic hierarchy concept. *Lancet Oncol.* 24 (7), e312–e322. doi:10.1016/s1470-2045(23)00186-9

Zhu, A. X., Kang, Y. K., Yen, C. J., Finn, R. S., Galle, P. R., Llovet, J. M., et al. (2019). Ramucirumab after sorafenib in patients with advanced hepatocellular carcinoma and increased α-fetoprotein concentrations (REACH-2): a randomised, double-blind, placebo-controlled, phase 3 trial. *Lancet Oncol.* 20 (2), 282–296. doi:10.1016/S1470-2045(18)30937-9

frontiers | Frontiers in Pharmacology

TYPE Original Research
PUBLISHED 30 September 2022
DOI 10.3389/fphar.2022.998534



OPEN ACCESS

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SPECIALTY SECTION
This article was submitted to Gastrointestinal and Hepatic Pharmacology, a section of the journal Frontiers in Pharmacology

RECEIVED 29 July 2022
ACCEPTED 31 August 2022
PUBLISHED 30 September 2022

CITATION
Guan R, Yu C, Li S, Mei J, Wei W and Guo R (2022), A preliminary study on drug switching strategy for second-line therapy after combination treatment of tyrosine kinase inhibitors and immune checkpoint inhibitors for unresectable hepatocellular carcinoma.
*Front. Pharmacol.* 13:998534.
doi: 10.3389/fphar.2022.998534



# A preliminary study on drug switching strategy for second-line therapy after combination treatment of tyrosine kinase inhibitors and immune checkpoint inhibitors for unresectable hepatocellular carcinoma

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**Background:** Combination treatment with tyrosine kinase inhibitors (TKIs) and immune checkpoint inhibitors (ICIs) has been widely used in patients with unresectable hepatocellular carcinoma (uHCC). As no standard guidelines exist for second-line therapy after failure of combination treatment, this study aimed to determine a better drug-switching strategy.

**Methods:** A total of 785 patients with uHCC who initially received a combination treatment of TKIs and ICIs between January 2017 and December 2021 at our center were screened. After applying the inclusion and exclusion criteria, a total of 102 patients were included in the study. Based on drug switching strategy, patients were divided into a single drug-switching group (A group, n = 49) and a double drug-switching group (B group, *n* = 53). The comparative effectiveness between groups A and B was assessed based on treatment response and survival time. Second progression-free survival (SPFS) and overall survival (OS) were compared using the Kaplan-Meier method and log-rank test.

**Results:** Compared to group B, group A had a higher overall response rate (16.3% vs. 3.8%; $p = 0.0392$) and disease control rate (61.2% vs. 49.1%; $p = 0.238$).

**Abbreviations:** AEs, Adverse events; AFP, α-fetoprotein; ALP, Alkaline phosphatase; ALT, Alanine aminotransferase; AST, Aspartate aminotransferase; BCLC, Barcelona Clinic Liver Cancer stage; CR, Complete response; DCR, Disease control rate; HCC, Hepatocellular carcinoma; HR, Hazards ratio; ICIs, Immune checkpoint inhibitors; OS, Overall survival; ORR, Objective response rate; PD, Progressive disease; PFS, Progression-free survival; PIVKA-II, Protein induced by vitamin K absence or antagonist-II; PR, Partial response; SD, Stable disease; SPFS, Second progression-free survival; TB, Total bilirubin; TKIs, Tyrosine kinase inhibitors; uHCC, Unresectable hepatocellular carcinoma.

The median SPFS in group A was longer than that in group B (5.47 vs. 3.8 months; HR = 1.70, $p$ = 0.0176). In the second-line therapy, the inclusion of lenvatinib resulted in a better SPFS than other TKI treatments (5.53 vs. 2.83 months, $p$ = 0.0038).

**Conclusion:** After the failure of the combination treatment of TKIs and ICIs, single-drug switching significantly prolonged median SPFS in uHCC patients, and retaining lenvatinib resulted in the survival benefit of single-drug switching.

KEYWORDS

**hepatocellular carcinoma, combination treatment, tyrosine kinase inhibitor, immune checkpoint inhibitor, drug switching, second-line therapy**

## Introduction

Hepatocellular carcinoma (HCC) is one of the most frequent malignancies and the third leading cause of cancer deaths worldwide (Sung et al., 2021). Hepatectomy and liver transplantation are the most effective treatments for HCC. Because of its unobvious symptoms, many patients with HCC are diagnosed at advanced stages, and hence have a low survival rate (Yang and Heimbach, 2020). Systemic therapy, including systemic chemotherapy and local interventional therapy, is the predominant therapeutic modality for unresectable HCC (uHCC). A previous study has demonstrated that compared to the best supportive care, metronomic capecitabine was an alternative choice to sorafenib with better efficacy and safety (De Lorenzo et al., 2018). Local therapy also brings significant benefits to uHCC patients. Recently, a multi-center propensity score-matched analysis has confirmed that transarterial infusion chemotherapy with FOLFOX was an effective and safe therapy that improved the survival of advanced hepatocellular carcinoma (Li et al., 2021). Transarterial chemoembolization (TACE) is recommended as the standard therapy for HCC patients with BCLC stage B (Mei et al., 2021).

In recent years, with the progress in research and clinical application of targeted and immunotherapy drugs, the prognosis of patients with uHCC has significantly improved (Llovet et al., 2008; Kudo et al., 2018; Finn et al., 2020; Yau et al., 2022). However, less than 20% of patients with uHCC benefited from immune checkpoint inhibitors (ICIs) monotherapy (Rizzo et al., 2021). The role of ICI-based combinations warrants further evaluation, and it is exciting that better prognosis benefits were demonstrated with combination therapy of tyrosine kinase inhibitors (TKIs) and ICIs than with monotherapy (Cheng et al., 2020a). The IMbrave 150 study reported that atezolizumab plus bevacizumab could result in better overall survival (OS) and progression-free survival (PFS) in the Chinese subpopulation (Qin et al., 2021). Moreover, $^{90}$Yttrium transarterial radioembolization has an established synergism with atezolizumab plus bevacizumab treatment by enhancing antigen presentation and reducing the infiltration of immunosuppressive cells (Di Federico et al., 2022). The KEYNOTE 524 reported significant improvements with pembrolizumab plus lenvatinib, with an objective response rate (ORR) of 46% (Llovet et al., 2022). Camrelizumab combined with apatinib as the first-line therapy can significantly prolong PFS and OS in patients with advanced HCC when compared with sorafenib, and the independent data monitoring committee judged that the primary endpoint of the study met the protocol-preset superiority criteria (SHR-1210-III-310). Thus, combination treatment with TKIs and ICIs has been applied as a first-line treatment for patients in China (Zhao and Cai, 2021). Owing to tumor heterogeneity, tumor progression still occurs in patients with HCC receiving first-line treatment. Although there are some options for second-line treatment (Choi et al., 2020; Zhang et al., 2020), there is a lack of widely accepted guidelines for switching therapy.

To our knowledge, real-world outcomes of switching therapy and a comparison of its efficacy have not been reported. Based on real-world data from clinical practice, this study aimed to explore the effect of the mode of switching therapy on the prognosis of uHCC after first-line systemic therapy failure, and thus providing a reference for larger prospective clinical studies in the future to guide the complete treatment of HCC.

## Patients and methods

### Ethics statement

The Institutional Review Board of the Ethics Committee of Sun Yat-sen University Cancer Center approved this study (B2020-190-01). All procedures involving human participants were performed in accordance with the Declaration of Helsinki. Written informed consent was obtained from all patients for anonymized information published in this article.

TABLE 1 Initial combination treatment of the patients.

| Tyrosine kinase inhibitors | Immune checkpoint inhibitors | A group ($n$ = 49) | B group ($n$ = 53) |
|---|---|---|---|
| Apatinib | Camrelizumab | 2 | 2 |
| | Tislelizumab | 0 | 1 |
| | Toripalimab | 0 | 9 |
| | Keytruda | 1 | 0 |
| | Nivolumab | 0 | 1 |
| | Sintilimab | 0 | 1 |
| Lenvatinib | Camrelizumab | 1 | 0 |
| | Toripalimab | 21 | 7 |
| | Keytruda | 6 | 4 |
| | Nivolumab | 0 | 4 |
| | Sintilimab | 8 | 7 |
| | Durvalumab | 0 | 1 |
| Regorafenib | Sintilimab | 1 | 1 |
| | Keytruda | 0 | 3 |
| | Durvalumab | 1 | 0 |
| Sorafenib | Toripalimab | 4 | 6 |
| | Nivolumab | 2 | 0 |
| | Sintilimab | 1 | 7 |

uHCC: unresectable HCC.

## Study population

Patients with uHCC who received TKIs and ICIs at the Department of Liver Surgery of Sun Yat-sen University Cancer Center between January 2017 and December 2021 were included in this retrospective analysis. The inclusion criteria for patients were as follows: 1) aged 18–75 years; 2) diagnosed with uHCC according to the AASLD practice guidelines (Marrero et al., 2018); 3) Child-Pugh class A or B; 4) at least one measurable lesion based on the modified Response Evaluation Criteria in Solid Tumors (mRECIST) criteria (Llovet and Lencioni, 2020); and 5) switched to at least one systemic therapy drug after tumor progression. The exclusion criteria of the patients were as follows: 1) presence of other malignant tumors; 2) no response evaluation after switching therapy; 3) incomplete baseline and follow-up data; and 4) clinical trials participants. A total of 102 patients with HCC were included in this study based on the inclusion and exclusion criteria. The details of the initial combination treatment and second-line treatment for the 102 uHCC patients are listed in Tables 1 and 2, respectively. All the patients were classified into two groups: group A ($n$ = 49) and group B ($n$ = 53), based on the mode of switching therapy. The group A included uHCC patients who switched to one systemic therapy drug after tumor progression, while the group B included patients who switched to two systemic therapy drugs after tumor progression. A flowchart of the patient disposition process is shown in Figure 1.

## Treatment procedure

Patients with uHCC received a combination of ICIs and TKIs as initial treatment. TKIs including regorafenib, apatinib, sorafenib, and lenvatinib were administered orally once daily. The ICIs included PD-1 and PD-L1 inhibitors which were intravenously injected every 3 weeks. The initial doses of TKIs and ICIs used are listed in Supplementary Table S1. The interval between the initiation of ICIs and TKIs was less than 7 days. Combination treatment with ICIs and TKIs was continued until the occurrence of disease progression or intolerable toxicity. After tumor progression, the decision to switch drugs was based on resistance to TKIs and ICIs or liver function. All patients with uHCC underwent scheduled enhanced computed tomography or magnetic resonance imaging assessment every 2-3 months.

## Data collection and clinical outcomes

All baseline data before second-line treatment were retrieved from medical records and imaging examinations, including age, sex, Child-Pugh class, α-fetoprotein (AFP), protein induced by vitamin K absence or antagonist-II (PIVKA-II), albumin, total bilirubin (TB), etiology, Barcelona Clinic Liver Cancer stage (BCLC stage), alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), progressive, macroscopic portal vein invasion, portal hypertension, and extrahepatic metastases. Tumor response to treatment was defined as complete response (CR), partial response (PR), stable disease (SD), or progressive disease (PD), based on the mRECIST criteria.

Second progression-free survival (SPFS) and OS were the clinical outcomes of interest. SPFS was defined as the interval from the initiation of second-line treatment to tumor progression, while OS was measured from the initiation of second-line treatment to death. The secondary outcomes included objective response rate (ORR) and disease control rate (DCR). ORR was defined as achieving CR or PR, and DCR was defined as achieving CR, PR, or SD. Treatment-related adverse events (AEs) were evaluated using the National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE) version 4.0.

TABLE 2 Second-line treatment given to the patients.

| Tyrosine kinase inhibitors | Immune checkpoint inhibitors | A group ($n$ = 49) | B group ($n$ = 53) |
|---|---|---|---|
| Apatinib | Camrelizumab | 0 | 7 |
| | Toripalimab | 3 | 0 |
| | NA | 0 | 1 |
| Lenvatinib | Camrelizumab | 5 | 3 |
| | Toripalimab | 6 | 1 |
| | Keytruda | 4 | 1 |
| | Nivolumab | 2 | 0 |
| | Sintilimab | 13 | 8 |
| | Durvalumab | 3 | 0 |
| | Tislelizumab | 6 | 3 |
| | NA | 0 | 8 |
| Regorafenib | Camrelizumab | 1 | 0 |
| | Tislelizumab | 0 | 1 |
| | Durvalumab | 0 | 1 |
| | Sintilimab | 2 | 0 |
| | NA | 0 | 5 |
| Sorafenib | Camrelizumab | 0 | 1 |
| | Toripalimab | 2 | 0 |
| | Sintilimab | 1 | 1 |
| | NA | 0 | 3 |
| Bevacizumab | Atezolizumab | 0 | 8 |
| | Durvalumab | 0 | 1 |
| | Lenvatinib | 1 | 0 |
| | NA | 0 | 0 |

Abbreviation: uHCC: unresectable HCC.

## Statistical analysis

The baseline characteristics were compared between the different modes of switching therapy. Continuous variables with normal distribution were expressed as means and standard deviations and those with abnormal distribution were expressed as medians and interquartile ranges. Continuous variables were analyzed using an unpaired Student's t-test for parametric data and the Mann–Whitney rank sum test for non-parametric data. Categorical variables were compared using Pearson's chi-squared test or Fisher's exact test. The survival analysis between the different treatment groups was performed by plotting Kaplan-Meier curves and their differences were verified using the log-rank test. Univariate Cox regression analysis was used to identify survival-associated factors, which were sequentially subjected to multivariate Cox regression analysis to identify the independent prognostic factors. All statistical analyses were performed using the SPSS software (version 20.0), MedCalc (version 20.027), and R software (version 4.1.1). Two-sided $p$-values <0.05 were considered statistically significant.

# Results

## Patient characteristics

The clinical characteristics of the patients and therapy given are summarized in Table 3. The median age of the study population was 54 years old. The majority of the patients were Child-Pugh class A ($n$ = 91, 89.22%) and chronically

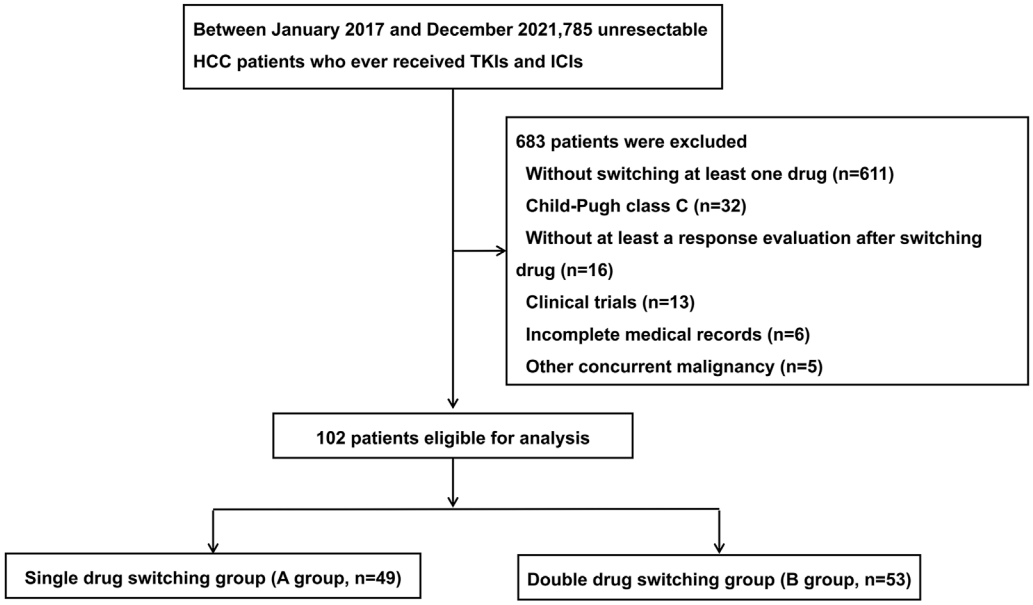


FIGURE 1
The flow chart of the disposition process of patients. HCC, hepatocellular carcinoma; TKIs, tyrosine kinase inhibitors (TKIs); ICIs, immune checkpoint inhibitors.

infected with the hepatitis B virus ($n$ = 92, 90.2%). Of the patients, 89.2% received other treatments, such as radiofrequency ablation, radiotherapy, hepatic artery infusion chemotherapy, transhepatic arterial chemotherapy, and embolization. Males were predominant ($n$ = 80, 78.43%) and 2/3rd of the patients were in BCLC stage C ($n$ = 76, 74.51%). The patients with extrahepatic metastases were approximately 60%. Almost half of the patients had macroscopic portal vein invasion ($n$ = 41, 40.2%) and single intrahepatic progression ($n$ = 42, 41.18%). In addition, 36.27% of patients had portal hypertension. The duration of first-line treatment and baseline characteristics were not significantly different between the groups ($p$ >0.05).

## Treatment response

The treatment responses are summarized in Table 4. Based on mRESIST, four patients had CR, six patients had PR, forty-six patients had SD and forty-six patients had PD. ORR and DCR were 9.8% and 54.9%, respectively. Notably, the ORR was higher in group A (16.3%) than in group B (3.8%) ($p$ = 0.0392). A higher DCR was observed in group A than in group B (61.2% vs. 49.1%; $p$ = 0.238). Collectively, the single drug switching strategy might provide clinical benefits to patients with uHCC.

## Comparison of the effectiveness of the switching modes

As shown in Figures 2A,B, the median SPFS was significantly longer in group A (5.47 months) than in group B (3.8 months) (HR = 1.70, 95%CI: 1.089–2.641, $p$ = 0.0176), while there was no significant difference in OS between group A and group B (HR = 1.12, 95%CI: 0.55–2.26, $p$ = 0.7556). The median OS in groups A and B were 20.7 and 21.6 months, respectively.

## Single drug switching extended second progression-free survival of patients with BCLC Stage A or B

A subgroup analysis was performed to identify the subset of patients who could benefit from a single drug-switching strategy. Interestingly, single drug switching strategy extended the SPFS of HCC patients with AFP<400 ng/ml (HR = 1.89, 95%CI: 1.01–3.55, $p$ = 0.0365), Child-Pugh class A (HR = 2.12, 95% CI: 1.32–3.41, $p$ = 0.0018), absence of macroscopic portal vein invasion (HR = 1.88, 95%CI: 1.05–3.35, $p$ = 0.0275), BCLC stage A or B (HR = 2.78, 95%CI: 1.04–7.45, $p$ = 0.0414), absence of extrahepatic metastasis (HR = 2.48, 95%CI: 1.20–5.14, $p$ = 0.0166), and single progression pattern (HR = 2.45, 95%CI:

TABLE 3 Clinicopathological characteristics of hepatocellular carcinoma patients.

| Characteristics | Total ($n$ = 102) | A group ($n$ = 49) | B group ($n$ = 53) | $p$-value |
|---|---|---|---|---|
| Age, years | 54 (43, 63)[a] | 53.9 ± 12.5[b] | 51 ± 12.9[b] | 0.194 |
| Male sex, n (%) | 80 (78.43) | 39 (79.59) | 41 (77.36) | 0.7841 |
| Child-Pugh class, n (%) | | | | 0.1003 |
| A | 91 (89.22) | 41 (83.67) | 50 (94.34) | |
| B | 11 (10.78) | 9 (16.33) | 3 (5.66) | |
| AFP, n (%) | | | | 0.2913 |
| ≥ 400 ng/ml | 44 (43.1) | 18 (36.7) | 26 (49.1) | |
| <400 ng/ml | 58 (56.9) | 31 (63.3) | 27 (50.9) | |
| PIVKA-II, n (%) | | | | 0.1049 |
| ≥1,000 mAU/mL | 47 (46.1) | 18 (36.7) | 29 (54.7) | |
| <1,000 mAU/mL | 55 (53.9) | 31 (63.3) | 24 (45.3) | |
| Albumin, median (IQR), g/dL | 4 (3.8, 4.4) | 4.1 (3.8, 4.4) | 4.1 (3.9, 4.5) | 0.671 |
| Total bilirubin, median (IQR), mg/dL | 13.3 (10, 19.7) | 13.5 (10.7, 21.2) | 13.1 (10, 17.8) | 0.567 |
| Etiology, n (%) | | | | 0.3848 |
| Yes | 92 (90.2) | 46 (93.88) | 46 (86.79) | |
| No | 10 (9.8) | 3 (6.12) | 7 (13.21) | |
| BCLC stage, n (%) | | | | 0.7329 |
| A | 3 (2.94) | 2 (4.08) | 1 (1.89) | |
| B | 23 (22.55) | 10 (20.41) | 13 (24.53) | |
| C | 76 (74.51) | 37 (75.51) | 39 (73.58) | |
| Macroscopic portal vein invasion, n (%) | 41 (40.2) | 19 (38.78) | 22 (41.51) | 0.7784 |
| ALT, median (IQR) | 34.65 (24.4, 55.4) | 28.8 (21.9, 52.5) | 43.3 (29,65.3) | 0.082 |
| AST, median (IQR) | 41.25 (30.9, 65.6) | 39.2 (29.2, 63.9) | 43.4 (33.3,75) | 0.325 |
| ALP, median (IQR) | 107.9 (76.4,148) | 100.5 (71.5, 138.9) | 110.8 (82, 165.6) | 0.190 |
| Progressive-pattern | | | | 0.3926 |
| Only extrahepatic progression | 28 (27.45) | 14 (28.57) | 14 (26.415) | |
| Only intrahepatic progression | 42 (41.18) | 17 (34.69) | 25 (47.17) | |
| Both | 32 (31.37) | 18 (36.73) | 14 (26.415) | |
| Extrahepatic metastases | 58 (56.86) | 29 (59.18) | 29 (54.72) | 0.6491 |
| Lymph node | 34 (33.33) | 16 (32.65) | 18 (33.9) | |
| Lung | 34 (33.33) | 11 (22.45) | 23 (43.4) | |
| Peritoneum | 10 (9.8) | 4 (8.16) | 6 (11.3) | |
| Bone | 9 (8.8) | 5 (10.2) | 4 (7.5) | |
| Others | 12 (14.7) | 4 (8.16) | 8 (15) | |
| Portal hypertension | 37 (36.27) | 19 (38.78) | 18 (33.96) | 0.6135 |
| Other treatments | | | | 0.8559 |
| With | 91 (89.2) | 44 (89.8) | 47 (88.7) | |
| Without | 11 (10.8) | 5 (10.2) | 6 (11.3) | |
| Time interval of drug switching (days) | 18 (9,28) | 18 (12,25) | 16 (7,29) | 0.6294 |
| Duration of first-line treatment (months) | 6.5 (4.3,11.4) | 8.2 (4.2,14.1) | 5.6 (4.3,8.4) | 0.132 |

[a]median (IQR).
[b]mean ± standard deviation.

1.40–4.27, $p$ = 0.0019). However, SPFS was not extended in patients with macroscopic portal vein invasion (Figure 3). No significant difference in OS was observed among the different subgroups (Figure 4). Collectively, the mode of single drug switching could extend SPFS in patients, especially in those without BCLC stage A or B.

## Lenvatinib increased the second progression-free survival in the single drug-switching group

We further divided group A into TKIs switching and ICIs switching groups. No significant difference was observed between

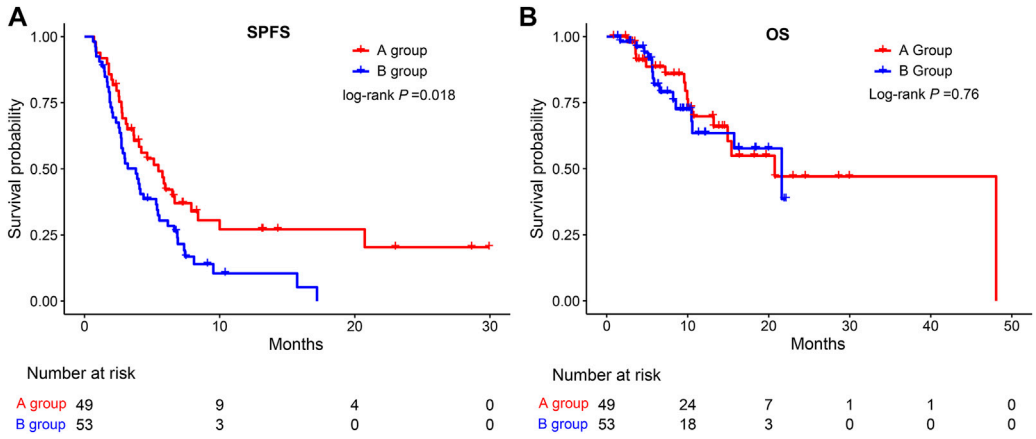


FIGURE 2
Kaplan-Meier survival curves for SPFS **(A)** and OS **(B)** of patients in the A group and the B group. SPFS, second progression-free survival; OS, overall survival.

the TKIs switching and B groups (HR = 0.63, 95%CI; 0.35–1.13, $p$ >0.05) (Figure 5A). However, compared to group B, the ICIs switching sub-group could significantly extend the SPFS (HR = 0.58, 95%CI: 0.36–0.95, $p$ = 0.029) (Figure 5B). The majority of uHCC patients in the ICIs switching group retained lenvatinib. Based on these results, we hypothesized that lenvatinib could be an important factor affecting the treatment efficacy. Thus, the effectiveness of lenvatinib treatment with other TKI treatments as second-line therapies was compared. As shown in Figure 6, lenvatinib treatment accounted for better SPFS than other TKI treatments (5.53 vs. 2.83 months, $p$ = 0.0038).

## Lenvatinib in the comprehensive treatment for unresectable hepatocellular carcinoma

In addition, the efficacy of lenvatinib as a first-line sequential treatment was investigated. As shown in Figure 7, patients who received lenvatinib as first-line therapy, compared to other TKIs treatments, could still benefit from retaining lenvatinib as the second-line treatment (5.97 vs. 2.73 months, $p$ = 0.0033). However, for those patients receiving other TKIs treatment as a first-line treatment, no survival benefit was reported between lenvatinib and other TKIs treatments in the second-line treatment (5.43 vs. 4.36 months, $p$ >0.05).

TABLE 4 Treatment response of patients.

| Evaluation (mRECIST) | Total | A group | B group |
|---|---|---|---|
| Complete response | 4 | 2 | 2 |
| Partial response | 6 | 6 | 0 |
| Stable disease | 46 | 22 | 24 |
| Progressive disease | 46 | 19 | 27 |
| Objective response rate[#] (%) | 9.8 | 16.3 | 3.8 |
| Disease control rate* (%) | 54.9 | 61.2 | 49.1 |
| Death | 31 | 16 | 15 |

Abbreviation: mRECIST, modified response evaluation criteria in solid tumors.
# Two-sided $p$-value = 0.0392.
* Two-sided $p$-value = 0.238.

## Safety analysis

As shown in Table 5, no AE-associated deaths were observed during the follow-up. The most common AEs were increased AST, followed by increased ALT, and pain in both groups. Seven (14.3%) and eleven (20.8%) patients in groups A and B experienced at least one grade 3/4 AE. Grade 3 AEs are severe, serious, or medically significant but not immediately life-threatening, requiring hospitalization or prolonged hospitalization and partial loss of self-care. Grade 4 AEs are life-threatening, which may lead to fatal consequences, and urgent intervention is required. The AEs in Groups A and B were manageable.

## Prognostic factors for second progression-free survival and overall survival

The results of univariate Cox regression analysis indicated that AFP≥400 (HR = 1.797, $p$ = 0.0116), BCLC stage C (HR = 1.959, $p$ = 0.0173), Child-Pugh class B (HR = 2.649, $p$ = 0.0049), extrahepatic metastasis (HR = 1.769, $p$ = 0.0165), PIVKA-II≥1,000 (HR = 1.874, $p$ = 0.0036), progression pattern (HR = 1.735, $p$ = 0.007), and switching to two systemic therapy drugs after tumor progression (HR = 1.722, $p$ = 0.0192) were potential prognostic biomarkers of

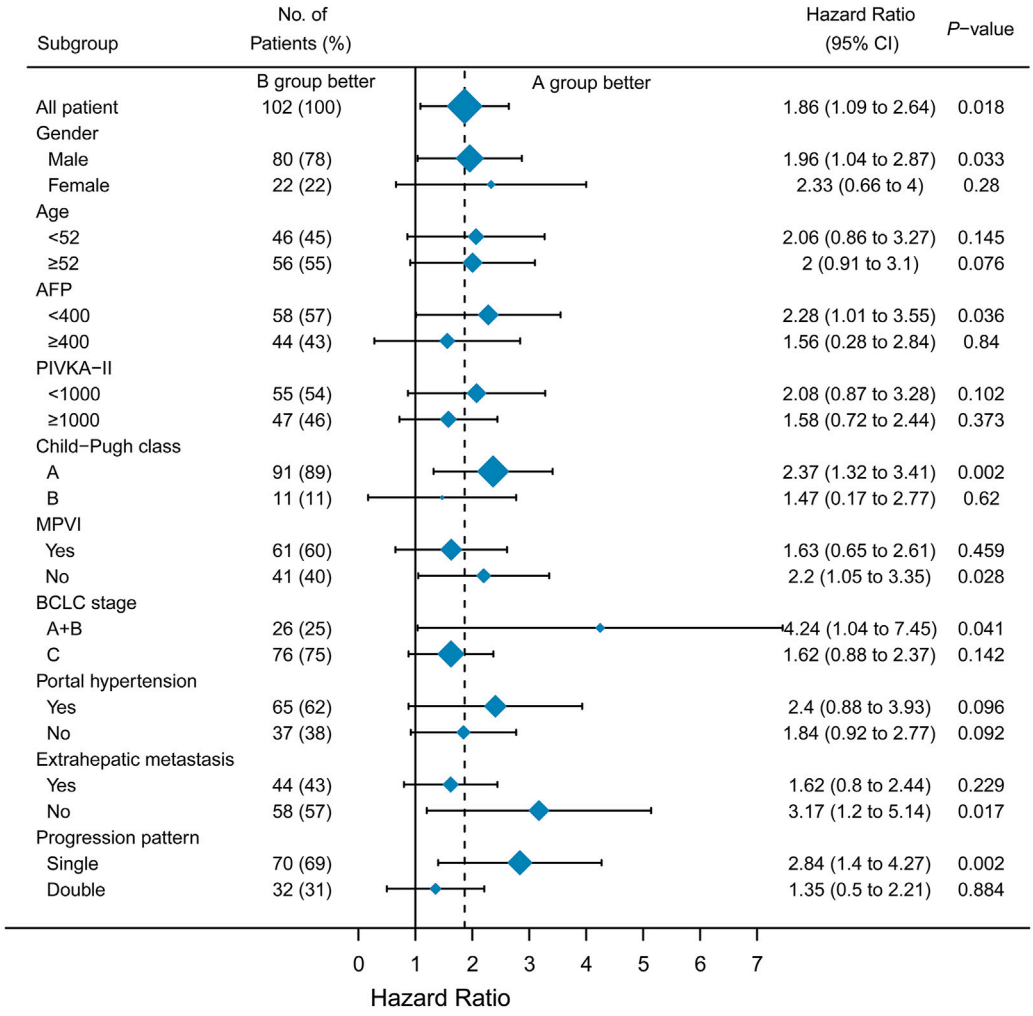


FIGURE 3
Subgroup analysis of second progression-free survival. MPVI, macroscopic portal vein invasion.

SPFS. The potentially predictive biomarkers were introduced into multivariate Cox regression analysis which confirmed that Child-Pugh class B (HR = 4.060, $p$ <0.001) and switching to two systemic therapy drugs after tumor progression (HR = 4.060, $p$ = 0.0123) were independent prognostic factors for SPFS (Table 6). In addition, extrahepatic metastasis (HR = 2.212, $p$ = 0.055), PIVKA-II≥1,000 (HR = 2.603, $P$= 0.0119), and progression pattern (HR = 2.684, $p$ <0.001) were potential prognostic biomarkers for OS. Further analysis indicated that PIVKA-II≥1,000 (HR = 2.651, $P$= 0.0118) was an adverse prognostic factor for OS (Table 7).

## Discussion

The treatment of uHCC is primarily based on systemic therapy. The age of patients undergoing combination treatment with TKIs and ICIs has decreased. There is abundant evidence to support that uHCC patient can benefit from a combination treatment of TKIs and ICIs (Cheng et al., 2020b). However, for HCC patients who progress on first-line combination treatment, many treatment options are available for subsequent therapies. Moreover, there is still a lack of generally accepted guidelines to guide second-line therapy after the progression of first-line combination treatment. There are two strategies for switching drugs in clinical practice: single drug switching (group A) and double drug switching (group B). This retrospective study aimed to evaluate and compare the effectiveness of two strategies of drug switching for patients with HCC who had failed combination treatment with TKIs and ICIs based on real-world data from clinical practice.

In our study, 102 patients with HCC were divided into groups A ($n$ = 49) and B ($n$ = 53). We observed a higher

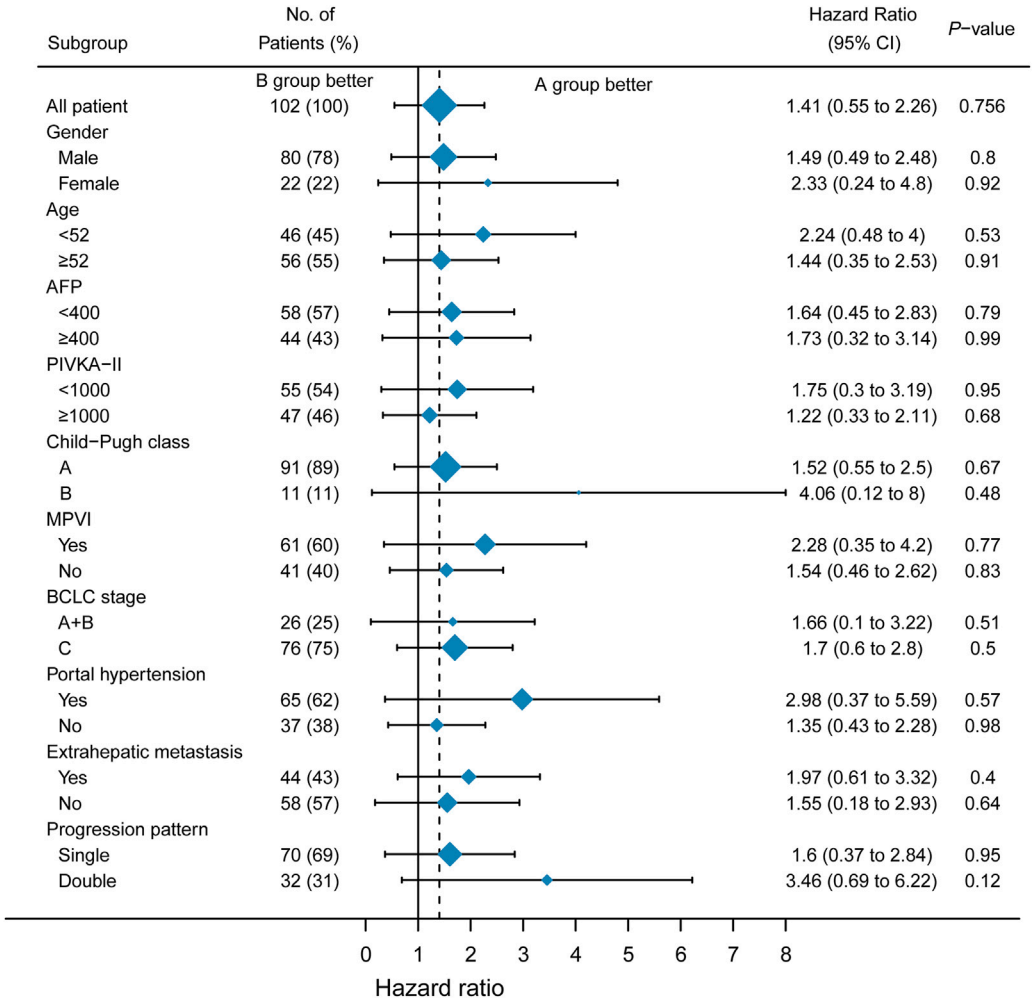


FIGURE 4
Subgroup analysis of second overall survival. MPVI, macroscopic portal vein invasion.

ORR (16.3%) and DCR (61.2%) in the group A. Further survival analysis indicated a significant difference in SPFS between groups A and B. Surprisingly, the median SPFS of group A was longer than that of group B (5.47 vs. 3.8 months, $p = 0.0176$). These data suggest that the median SPFS in our study was significantly extended compared to that of a previous study where the sequence ramucirumab for uHCC after TKI treatment (Amioka et al., 2021).

However, we observed no differences in the OS between groups A and B. The reason for this may be as follows. First, the follow-up time for SPFS was shorter and the sample size for SPFS was smaller than for OS. Our study's sample size and follow-up time might not be sufficient for OS calculation. Second, OS might be affected by subsequent treatment and does not directly reflect the true efficacy of switching therapy. After switching therapy, patients with uHCC may receive other subsequent treatments, such as interventional therapy and radiotherapy. We did not observe a significant difference in OS between groups A and B.

We further analyzed which subgroup of patients could benefit from a single drug switch and double drug switch. In the subgroup analysis, we found that HCC patients with AFP<400, Child-Pugh class A, without macroscopic portal vein invasion, BCLC stage A or B, without extrahepatic metastasis, and a single progression pattern could benefit from the single drug switching strategy. In our study, Child-Pugh class A was associated with a better prognosis. A previous study demonstrated that uHCC patients with Child-Pugh class A could receive a sufficient relative dose intensity of lenvatinib, which sequentially affected the objective response (Sasaki et al., 2019). AFP level is used for the diagnosis of HCC. Previous studies have shown that there is a close relationship between AFP levels and response to comprehensive treatment (Chau et al., 2018). Consistent with a previous study, AFP < 400 ng/ml could

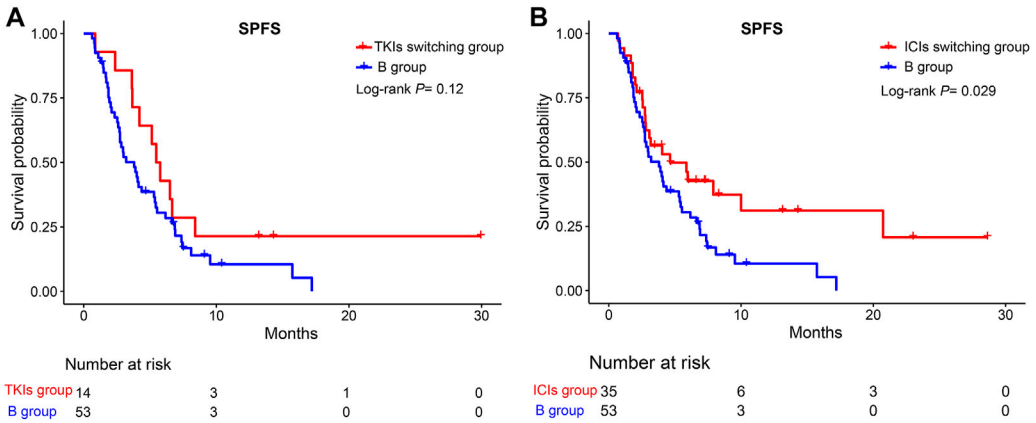


FIGURE 5
Kaplan-Meier curves for SPFS of patients in the TKIs switching group, ICIs switching group, and the B group. **(A)** TKIs switching group vs. B group; **(B)** ICIs switching group vs. B group. SPFS, second progression-free survival; TKIs, tyrosine kinase inhibitors; ICIs, immune checkpoint inhibitors.

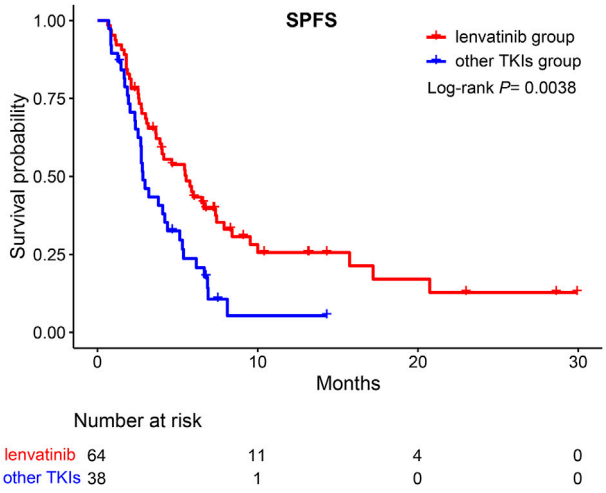


FIGURE 6
Kaplan-Meier curves for SPFS of patients moving to second-line therapy. SPFS, second progression-free survival; TKIs, tyrosine kinase inhibitors.

predict the response to a single drug-switching strategy (Regmi et al., 2021). BCLC staging is a generally acknowledged system for the treatment of HCC (Reig et al., 2022). As for the single progression pattern, the reason it could benefit from single drug switching may be associated with the microenvironment. The sole progression pattern indicates that one of the tumor sites may be curbed or eradicated. However, this hypothesis requires further investigation. Macroscopic portal vein invasion and extrahepatic metastasis are the parameters of BCLC stage C. Mei et al. (2021) demonstrated that HCC patients with main portal vein tumor thrombus or extrahepatic metastasis could not benefit most from hepatic artery infusion chemotherapy plus lenvatinib combination therapy. In our study, these results indicate that the single-drug switching strategy might be suitable for patients with BCLC stage A or B. BCLC stage C indicates a more malignant tumor. As a result, compared with uHCC patients with stage A or B disease, patients with macroscopic portal vein invasion or extrahepatic metastasis seemed to be more inclined to progress, leading to a worse survival prognosis. Collectively, the mode of single drug switching could extend SPFS in patients, especially in those with BCLC stage A or B.

In our study, patients could benefit from single-drug switching rather than double-drug switching. To explain the reasons for this result, we further divided group A into TKIs switching and ICIs switching groups. Surprisingly, compared with group B, the ICIs switching group could significantly extend the SPFS. However, no significant difference was reported between the TKIs switching and the B group. Both uHCC patients in the ICIs switching group and B group switched ICIs after tumor progression. Why could the former group extend the SPFS? We found that the majority of uHCC patients in the ICIs switching group retained lenvatinib. Moreover, for second-line therapy, lenvatinib treatment accounted for a better SPFS than other TKI treatments (5.53 vs. 2.83 months, $p = 0.0038$). This result further confirms our hypothesis. The REFLECT clinical trial indicated that the overall survival time of the lenvatinib group was not inferior to the sorafenib group (Kudo et al., 2018). Further studies indicated that, compared with sorafenib, lenvatinib exhibited stronger inhibitory activity targeting the fibroblast growth factor receptor (Tohyama et al., 2014). Shi et al. (2021) found that lenvatinib may be a suitable second-line treatment for uHCC patients who

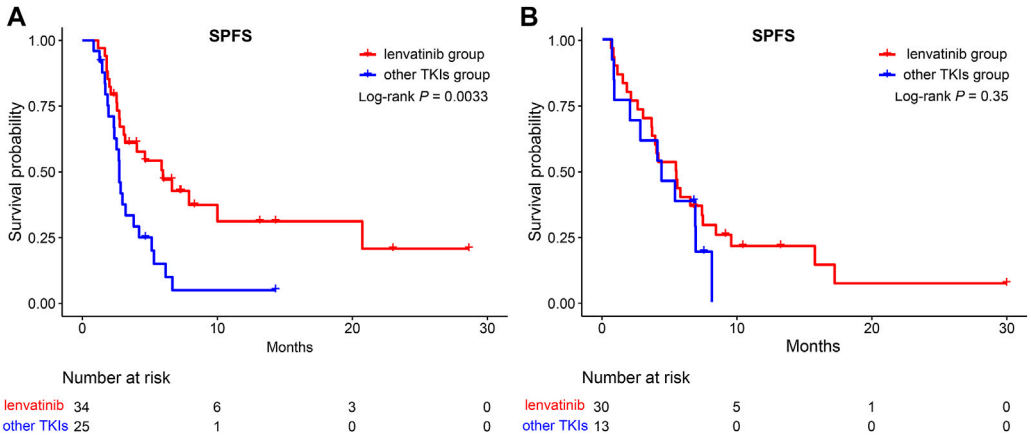


FIGURE 7
Kaplan-Meier curves for SPFS of patients in the lenvatinib group and the other TKIs group. **(A)** A subgroup of patients after lenvatinib as first-line therapy; **(B)** A subgroup of patients after other TKIs as first-line therapy. SPFS, second progression-free survival; TKIs, tyrosine kinase inhibitors.

TABLE 5 Treatment-related adverse events.

| Adverse events | Any grade | | | Grade 3/4 | | |
|---|---|---|---|---|---|---|
| | A group ($n$ = 49) | B group ($n$ = 53) | $p$-value | A group ($n$ = 49) | B group ($n$ = 53) | $p$-value |
| Treatment-related AEs, n (%) | | | | | | |
| Rash | 2 (4) | 6 (11.3) | 0.3221 | 0 (0) | 1 (1.9) | 1 |
| Pruritus | 2 (4) | 2 (3.8) | 1 | 0 (0) | 1 (1.9) | 1 |
| Pain | 11 (22) | 14 (26.4) | 0.6541 | 2 (4.1) | 4 (0) | 0.4574 |
| Fever | 2 (4) | 4 (7.5) | 0.7474 | 0 (0) | 0 (0) | 1 |
| Diarrhea | 6 (14) | 4 (7.5) | 0.6427 | 0 (0) | 0 (0) | 1 |
| Fatigue | 4 (8) | 3 (5.7) | 0.9143 | 0 (0) | 0 (0) | 1 |
| Nausea | 2 (4) | 3 (5.7) | 0.7122 | 0 (0) | 0 (0) | 1 |
| Decreased appetite | 5 (10) | 6 (11.3) | 0.8559 | 0 (0) | 0 (0) | 1 |
| Cough | 6 (12) | 4 (7.5) | 0.6427 | 2 (4.1) | 1 (1.9) | 0.9450 |
| Edema periphera | 3 (6) | 1 (1.8) | 0.5548 | 1 (2.0) | 0 (0) | 0.4804 |
| Hypothyroidism | 2 (4) | 3 (5.7) | 1 | 0 (0) | 0 (0) | 1 |
| Hyperthyroidism | 0 (0) | 1 (1.9) | 1 | 0 (0) | 0 (0) | 1 |
| Laboratory-related AEs, n (%) | | | | | | |
| White blood cell count decreased | 3 (6) | 3 (5.7) | 0.9211 | 0 (0) | 0 (0) | 1 |
| Hemoglobin decreased | 4 (8) | 8 (15.1) | 0.4366 | 0 (0) | 2 (3.8) | 0.4958 |
| Platelet count decreased | 7 (14.3) | 6 (11.3) | 0.6537 | 1 (2.0) | 2 (3.8) | 0.6048 |
| Neutropenia | 1 (2) | 2 (3.8) | 0.6048 | 1 (2.0) | 0 (0) | 0.9553 |
| Alanine aminotransferase increased | 15 (30.6) | 21 (39.6) | 0.3414 | 1 (2.0) | 0 (0) | 0.4804 |
| Aspertate aminotransferase increased | 18 (36.7) | 28 (52.8) | 0.1026 | 1 (2.0) | 3 (5.7) | 0.6669 |
| Total bilirubin increased | 9 (18.4) | 8 (15.1) | 0.6577 | 2 (4.1) | 0 (0) | 0.2283 |
| Albumin decreased | 9 (18.4) | 10 (18.9) | 0.9483 | 0 (0) | 0 (0) | 1 |
| Creatinine increased | 1 (2) | 2 (3.8) | 0.6048 | 0 (0) | 0 (0) | 1 |

Abbreviation: AEs, adverse events.

TABLE 6 Univariable and multivariable Cox regression analyses for second progression-free survival.

| Characteristic | Univariate analysis | | Multivariable analysis | |
|---|---|---|---|---|
| | HR (95% CI) | *P* | HR (95% CI) | *P* |
| Age (</≥52) | 0.969 (0.620–1.515) | 0.89 | | |
| Gender, (female/male) | 1.002 (0.597–1.680) | 0.995 | | |
| AFP (ng/ml), (</≥400) | 1.797 (1.140–2.833) | 0.0116 | 1.462 (0.817–2.617) | 0.201 |
| PIVKA-II, (mAU/ml), (</≥1,000) | 1.957 (1.246–3.074) | 0.0036 | 1.325 (0.753–2.330) | 0.329 |
| Child-Pugh (A/B) | 2.649 (1.344–5.224) | 0.0049 | 4.052 (1.806–9.094) | 0.0007 |
| BCLC (A + B/C) | 1.959 (1.126–3.408) | 0.0173 | 0.885 (0.395–1.979) | 0.765 |
| Extrahepatic metastasis (no/yes) | 1.757 (1.108–2.785) | 0.0165 | 1.892 (0.926–3.865) | 0.0802 |
| Macroscopic portal vein invasion (no/yes) | 1.229 (0.783–1.929) | 0.371 | | |
| Portal hypertension (no/yes) | 1.126 (0.706–1.793) | 0.619 | | |
| Progressive-pattern (single/both) | 1.897 (1.191–3.019) | 0.007 | 1.644 (0.988–2.736) | 0.056 |
| Drug switching group (A group/B group) | 1.722 (1.093–2.712) | 0.0192 | 1.844 (1.142–2.978) | 0.0123 |

TABLE 7 Univariable and multivariable Cox regression analyses for overall survival.

| Characteristic | Univariate analysis | | Multivariable analysis | |
|---|---|---|---|---|
| | HR (95% CI) | *P* | HR (95% CI) | *P* |
| Age (</≥52) | 0.98 (0.476–2.020) | 0.957 | | |
| Gender, (female/male) | 0.938 (0.402–2.188) | 0.883 | | |
| AFP (ng/ml), (</≥400) | 1.029 (0.493–2.146) | 0.94 | | |
| PIVKA-II, (mAU/ml), (</≥1,000) | 2.603 (1.235–5.491) | 0.0119 | 2.651 (1.242–5.662) | 0.0118 |
| Child-Pugh (A/B) | 1.910 (0.663–5.500) | 0.23 | | |
| BCLC (A + B/C) | 1.851 (0.707–4.845) | 0.21 | | |
| Extrahepatic metastasis (no/yes) | 2.212 (0.984–4.971) | 0.055 | 1.889 (0.786–4.536) | 0.155 |
| Macroscopic portal vein invasion (no/yes) | 0.852 (0.397–1.831) | 0.682 | | |
| Portal hypertension (no/yes) | 0.69 (0.307–1.554) | 0.371 | | |
| Progressive-pattern (single/both) | 2.826 (1.375–5.809) | 0.005 | 2.072 (0.954–4.501) | 0.066 |
| Drug switching group (A group/B group) | 1.121 (0.545–2.306) | 0.756 | | |

progressed on sorafenib by regulating FGFR4-ERK signaling. Apatinib is a small-molecule tyrosine kinase inhibitor that selectively inhibits the activity of VEGFR-2 (Tian et al., 2011). Moreover, a previous study indicated that lenvatinib had immunomodulatory activity, which contributed to the antitumor effect of lenvatinib and enhanced the synergistic effect with the anti-PD-1 antibody (Kimura et al., 2018). Moreover, Chen et al. demonstrated that lenvatinib could reduce the expression of PD-L1 in HCC and regulate T-cell differentiation by blocking FGFR4 to improve anti-PD-1 efficacy (Yi et al., 2021). Collectively, retaining lenvatinib accounted for the survival benefits of single-drug switching, especially in SPFS. However, lenvatinib led to better SPFS, but did not translate into OS benefits. The use of longer SPFS with lenvatinib to enable patients to obtain longer OS benefits still needs to be explored by oncologists.

Further analysis indicated that for those patients who selected lenvatinib as the first-line treatment, compared to other TKIs treatment, they could still benefit from retaining lenvatinib as the second-line treatment (5.97 vs. 2.73 months, $p = 0.0033$). However, for patients who selected other TKIs as the first-line treatment, no survival benefit was reported between lenvatinib and other TKIs treatments. Chen et al. retrospectively analyzed 26 cases of advanced uHCC from October 2018 to October 2019 in the real world in China and found that lenvatinib combined with the PD-1 antibody was expected to further improve the prognosis of patients who progressed on lenvatinib (Chen

et al., 2020). Thus, lenvatinib should be used for the comprehensive treatment of uHCC. However, high-quality randomized controlled studies are required to validate this conclusion.

In the prognostic factor analysis, the Child-Pugh class and drug-switching strategy were identified as independent prognostic factors for SPFS. The Child-Pugh class is an evaluation system for liver reserve function, including five parameters (Kok and Abraldes, 2019). In our study, Child-Pugh class A could predict better SPFS, and HCC patients with Child-Pugh class A could obtain a longer SPFS benefit from the single drug switching strategy. The reason for this might be that HCC patients with Child-Pugh class A could better tolerate the combination therapy's toxicity. Moreover, a PIVKA-II>1,000 was regarded as an adverse prognostic factor for OS. Another prognostic factor is the drug-switching strategy. Based on the results of the comparison of the two drug-switching strategies in clinical practice, we found that single-drug switching could extend the SPFS. PIVKA-II is produced because of the incomplete carboxylation of amino acid residues (Liebman et al., 1984). What is clear is that PIVKA-II is not only a diagnostic predictor but also a prognostic predictor of liver cancer (Yang et al., 2021). PIVKA-II exhibited stronger mitogenic capacity and migratory activity during angiogenesis in HCC patients (Bertino et al., 2010).

As for safety, consistent with a previous study, toxicities were manageable with no unexpected safety signals (Mo et al., 2021). No AE-associated death was observed during follow-up, and the most common AEs were damage to liver function. Dose adjustments of TKIs and ICIs accounted for safety in the present study. In our study, the percentages of interruption and dose reduction in groups A and B were 30% and 35%, respectively. Half of the routine dosage or weekends-off administration of lenvatinib (Iwamoto et al., 2020) was the primary method of dose adjustment.

We acknowledge the potential limitations of this study. First, a selection bias was unavoidable because this was a retrospective study. Liver function was worse in group A than in group B, and it was positively correlated with survival rate. However, the survival analysis indicated that the treatment response and SPFS of group A were better than those of group B. The potential selection bias worked unfavorably against the single-drug switching strategy, leading to an opposite result. Secondly, one hundred and two patients with uHCC were included in our study. The sample size was small, and the observation period was short. All included patients were Asian, and their data were obtained from a single Chinese institute. A single drug-switching strategy might be beneficial only to the Asian population. A great amount of evidence has demonstrated that the carcinogenic factors of patients with HCC in Asia and the West are different, which limits the ability to draw general conclusions from the results (Marengo et al., 2016). Collectively, our conclusion requires further confirmation by a large international multicenter clinical study in the future. Third, confounding factors are one of the limitations. We defined drug-switching strategies for second-line therapy after combination treatment with TKIs and ICIs, but the optional treatment for HCC patients lacks clear guidelines. Subsequent treatments after first-line treatment were not chosen in a randomized manner. Thus, the therapeutic molecules used in the second line might vary between groups A and B, which influenced the uniformity of the treatment procedure. Such division of patients into different groups may bring about a certain degree of heterogeneity; thus, this was a preliminary study on a drug-switching strategy for second-line therapy after combination treatment with tyrosine kinase inhibitors and immune checkpoint inhibitors for unresectable hepatocellular carcinoma. The findings of this study should be further validated using higher-level randomized controlled trials. Finally, lenvatinib was the main TKIs used in combination with ICIs in our study. Thus, the value of other TKIs, such as sorafenib and regorafenib, in combination treatment should be further investigated.

## Conclusion

After combination treatment with TKIs and ICIs failure, single-drug switching significantly prolonged the median SPFS in uHCC patients, and retaining lenvatinib accounted for the survival benefit brought by single-drug switching.

## Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding authors.

## Ethics statement

The studies involving human participants were reviewed and approved by The Institutional Review Board of the Ethics Committee of Sun Yat-Sen University Cancer Center approved this study (B2020-190-01). The patients/participants provided their written informed consent to participate in this study.

## Author contributions

RG: data curation; formal analysis; software; methodology; writing-original draft; writing-review and editing. CY: formal analysis; investigation; software; writing-original draft. JM: project administration; visualization; writing-review and editing. SL: investigation; supervision; visualization; writing-review and

editing. WW: investigation; supervision; visualization; writing-review and editing. RoG: conceptualization; funding acquisition; project administration; writing-review and editing.

## Funding

This study was supported by the National Natural Science Foundation of China (No. 82172579, No. 81871985), Natural Science Foundation of Guangdong Province (No. 2018A0303130098, No. 2017A030310203), Science and Technology Planning Project of Guangdong Province (No. 2017A020215112), and Medical Scientific Research Foundation of Guangdong Province (No. A2017477); Science and Technology Planning Project of Guangzhou (No. 201903010017, No. 201904010479); and Clinical Trials Project (5010 Project) of Sun Yat-Sen University (No. 5010-2017009).

## Acknowledgments

The authors thank all the patients who participated in this study.

## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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## Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphar.2022.998534/full#supplementary-material

## References

Amioka, K., Kawaoka, T., Ogawa, Y., Kikukawa, C., Naruto, K., Yoshikawa, Y., et al. (2021). Comparison of the clinical outcome of ramucirumab for unresectable hepatocellular carcinoma with that of prior tyrosine kinase inhibitor therapy. *Oncology* 99, 327–335. doi:10.1159/000514315

Bertino, G., Ardiri, A. M., Calvagno, G. S., Bertino, N., and Boemi, P. M. (2010). Prognostic and diagnostic value of des-gamma-carboxy prothrombin in liver cancer. *Drug News Perspect.* 23, 498–508. doi:10.1358/dnp.2010.23.8.1444236

Chau, I., Park, J. O., Ryoo, B. Y., Yen, C. J., Poon, R., Pastorelli, D., et al. (2018). Alpha-fetoprotein kinetics in patients with hepatocellular carcinoma receiving ramucirumab or placebo: An analysis of the phase 3 REACH study. *Br. J. Cancer* 119, 19–26. doi:10.1038/s41416-018-0103-0

Chen, Y., Zhang, L., Ge, N., Wang, Y., and Ren, Z. (2020). Clinical effect of sequential therapy of LEN combination with anti-PD1 antibody in uHCC patients who progressed on LEN treatment: A real-world data in China. *J. Clin. Oncol.* 38, e16654–e16654. doi:10.1200/JCO.2020.38.15_suppl.e16654

Cheng, A. L., Hsu, C., Chan, S. L., Choo, S. P., and Kudo, M. (2020). Challenges of combination therapy with immune checkpoint inhibitors for hepatocellular carcinoma. *J. Hepatol.* 72, 307–319. doi:10.1016/j.jhep.2019.09.025

Cheng, S., Chen, M., Cai, J., Sun, J., Guo, R., Bi, X., et al. (2020). Chinese expert consensus on multidisciplinary diagnosis and treatment of hepatocellular carcinoma with portal vein tumor thrombus (2018 edition). *Liver Cancer* 9, 28–40. doi:10.1159/000503685

Choi, W. M., Choi, J., Lee, D., Shim, J. H., Lim, Y. S., Lee, H. C., et al. (2020). Regorafenib versus nivolumab after sorafenib failure: Real-world data in patients with hepatocellular carcinoma. *Hepatol. Commun.* 4, 1073–1086. doi:10.1002/hep4.1523

De Lorenzo, S., Tovoli, F., Barbera, M. A., Garuti, F., Palloni, A., Frega, G., et al. (2018). Metronomic capecitabine vs. best supportive care in child-pugh B hepatocellular carcinoma: A proof of concept. *Sci. Rep.* 8, 9997. doi:10.1038/s41598-018-28337-6

Di Federico, A., Rizzo, A., Carloni, R., De Giglio, A., Bruno, R., Ricci, D., et al. (2022). Atezolizumab-bevacizumab plus Y-90 TARE for the treatment of hepatocellular carcinoma: Preclinical rationale and ongoing clinical trials. *Expert Opin. Investig. Drugs* 31, 361–369. doi:10.1080/13543784.2022.2009455

Finn, R. S., Ryoo, B. Y., Merle, P., Kudo, M., Bouattour, M., Lim, H. Y., et al. (2020). Pembrolizumab as second-line therapy in patients with advanced hepatocellular carcinoma in KEYNOTE-240: A randomized, double-blind, phase III trial. *J. Clin. Oncol.* 38, 193–202. doi:10.1200/JCO.19.01307

Iwamoto, H., Suzuki, H., Shimose, S., Niizeki, T., Nakano, M., Shirono, T., et al. (2020). Weekends-off lenvatinib for unresectable hepatocellular carcinoma improves therapeutic response and tolerability toward adverse events. *Cancers (Basel)* 12, E1010. doi:10.3390/cancers12041010

Kimura, T., Kato, Y., Ozawa, Y., Kodama, K., Ito, J., Ichikawa, K., et al. (2018). Immunomodulatory activity of lenvatinib contributes to antitumor activity in the Hepa1-6 hepatocellular carcinoma model. *Cancer Sci.* 109, 3993–4002. doi:10.1111/cas.13806

Kok, B., and Abraldes, J. G. (2019). Child-pugh classification: Time to abandon? *Semin. Liver Dis.* 39, 96–103. doi:10.1055/s-0038-1676805

Kudo, M., Finn, R. S., Qin, S., Han, K. H., Ikeda, K., Piscaglia, F., et al. (2018). Lenvatinib versus sorafenib in first-line treatment of patients with unresectable hepatocellular carcinoma: A randomised phase 3 non-inferiority trial. *Lancet* 391, 1163–1173. doi:10.1016/S0140-6736(18)30207-1

Li, S., Mei, J., Wang, Q., Shi, F., Liu, H., Zhao, M., et al. (2021). Transarterial infusion chemotherapy with FOLFOX for advanced hepatocellular carcinoma: A multi-center propensity score matched analysis of real-world practice. *Hepatobiliary Surg. Nutr.* 10, 631–645. doi:10.21037/hbsn.2020.03.14

Liebman, H. A., Furie, B. C., Tong, M. J., Blanchard, R. A., Lo, K. J., Lee, S. D., et al. (1984). Des-gamma-carboxy (abnormal) prothrombin as a serum marker of primary hepatocellular carcinoma. *N. Engl. J. Med.* 310, 1427–1431. doi:10.1056/NEJM198405313102204

Llovet, J. M., Castet, F., Heikenwalder, M., Maini, M. K., Mazzaferro, V., Pinato, D. J., et al. (2022). Immunotherapies for hepatocellular carcinoma. *Nat. Rev. Clin. Oncol.* 19, 151–172. doi:10.1038/s41571-021-00573-2

Llovet, J. M., and Lencioni, R. (2020). mRECIST for HCC: Performance and novel refinements. *J. Hepatol.* 72, 288–306. doi:10.1016/j.jhep.2019.09.026

Llovet, J. M., Ricci, S., Mazzaferro, V., Hilgard, P., Gane, E., Blanc, J. F., et al. (2008). Sorafenib in advanced hepatocellular carcinoma. *N. Engl. J. Med.* 359, 378–390. doi:10.1056/NEJMoa0708857

Marengo, A., Rosso, C., and Bugianesi, E. (2016). Liver cancer: Connections with obesity, fatty liver, and cirrhosis. *Annu. Rev. Med.* 67, 103–117. doi:10.1146/annurev-med-090514-013832

Marrero, J. A., Kulik, L. M., Sirlin, C. B., Zhu, A. X., Finn, R. S., Abecassis, M. M., et al. (2018). Diagnosis, staging, and management of hepatocellular carcinoma: 2018 practice guidance by the American association for the study of liver diseases. *Hepatology* 68, 723–750. doi:10.1002/hep.29913

Mei, J., Tang, Y. H., Wei, W., Shi, M., Zheng, L., Li, S. H., et al. (2021). Hepatic arterial infusion chemotherapy combined with PD-1 inhibitors plus lenvatinib versus PD-1 inhibitors plus lenvatinib for advanced hepatocellular carcinoma. *Front. Oncol.* 11, 618206. doi:10.3389/fonc.2021.618206

Mo, D. C., Luo, P. H., Huang, S. X., Wang, H. L., and Huang, J. F. (2021). Safety and efficacy of pembrolizumab plus lenvatinib versus pembrolizumab and lenvatinib monotherapies in cancers: A systematic review. *Int. Immunopharmacol.* 91, 107281. doi:10.1016/j.intimp.2020.107281

Qin, S., Ren, Z., Feng, Y. H., Yau, T., Wang, B., Zhao, H., et al. (2021). Atezolizumab plus bevacizumab versus sorafenib in the Chinese subpopulation with unresectable hepatocellular carcinoma: Phase 3 randomized, open-label IMbrave150 study. *Liver Cancer* 10, 296–308. doi:10.1159/000513486

Regmi, P., Hu, H. J., Lv, T. R., Paudyal, A., Sah, R. B., Ma, W. J., et al. (2021). Efficacy and safety of sorafenib plus hepatic arterial infusion chemotherapy for advanced hepatocellular carcinoma. *Surg. Oncol.* 39, 101663. doi:10.1016/j.suronc.2021.101663

Reig, M., Forner, A., Rimola, J., Ferrer-Fabrega, J., Burrel, M., Garcia-Criado, A., et al. (2022). BCLC strategy for prognosis prediction and treatment recommendation: The 2022 update. *J. Hepatol.* 76, 681–693. doi:10.1016/j.jhep.2021.11.018

Rizzo, A., Ricci, A. D., Gadaleta-Caldarola, G., and Brandi, G. (2021). First-line immune checkpoint inhibitor-based combinations in unresectable hepatocellular carcinoma: Current management and future challenges. *Expert Rev. Gastroenterol. Hepatol.* 15, 1245–1251. doi:10.1080/17474124.2021.1973431

Sasaki, R., Fukushima, M., Haraguchi, M., Miuma, S., Miyaaki, H., Hidaka, M., et al. (2019). Response to lenvatinib is associated with optimal RelativeDose intensity in hepatocellular carcinoma: Experience in clinical settings. *Cancers (Basel)* 11, E1769. doi:10.3390/cancers11111769

Shi, T., Iwama, H., Fujita, K., Kobara, H., Nishiyama, N., Fujihara, S., et al. (2021). Evaluating the effect of lenvatinib on sorafenib-resistant hepatocellular carcinoma cells. *Int. J. Mol. Sci.* 22, 13071. doi:10.3390/ijms222313071

Sung, H., Ferlay, J., Siegel, R. L., Laversanne, M., Soerjomataram, I., Jemal, A., et al. (2021). Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *Ca. Cancer J. Clin.* 71, 209–249. doi:10.3322/caac.21660

Tian, S., Quan, H., Xie, C., Guo, H., Lu, F., Xu, Y., et al. (2011). YN968D1 is a novel and selective inhibitor of vascular endothelial growth factor receptor-2 tyrosine kinase with potent activity *in vitro* and *in vivo*. *Cancer Sci.* 102, 1374–1380. doi:10.1111/j.1349-7006.2011.01939.x

Tohyama, O., Matsui, J., Kodama, K., Hata-Sugi, N., Kimura, T., Okamoto, K., et al. (2014). Antitumor activity of lenvatinib (e7080): An angiogenesis inhibitor that targets multiple receptor tyrosine kinases in preclinical human thyroid cancer models. *J. Thyroid. Res.* 2014, 638747. doi:10.1155/2014/638747

Yang, J. D., and Heimbach, J. K. (2020). New advances in the diagnosis and management of hepatocellular carcinoma. *BMJ* 371, m3544. doi:10.1136/bmj.m3544

Yang, Y., Li, G., Lu, Z., Liu, Y., Kong, J., and Liu, J. (2021). Progression of prothrombin induced by vitamin K absence-II in hepatocellular carcinoma. *Front. Oncol.* 11, 726213. doi:10.3389/fonc.2021.726213

Yau, T., Park, J. W., Finn, R. S., Cheng, A. L., Mathurin, P., Edeline, J., et al. (2022). Nivolumab versus sorafenib in advanced hepatocellular carcinoma (CheckMate 459): A randomised, multicentre, open-label, phase 3 trial. *Lancet. Oncol.* 23, 77–90. doi:10.1016/S1470-2045(21)00604-5

Yi, C., Chen, L., Lin, Z., Liu, L., Shao, W., Zhang, R., et al. (2021). Lenvatinib targets FGF receptor 4 to enhance antitumor immune response of anti-programmed cell death-1 in HCC. *Hepatology* 74, 2544–2560. doi:10.1002/hep.31921

Zhang, X. H., Cao, M. Q., Li, X. X., and Zhang, T. (2020). Apatinib as an alternative therapy for advanced hepatocellular carcinoma. *World J. Hepatol.* 12, 766–774. doi:10.4254/wjh.v12.i10.766

Zhao, H. T., and Cai, J. Q. (2021). Chinese expert consensus on neoadjuvant and conversion therapies for hepatocellular carcinoma. *World J. Gastroenterol.* 27, 8069–8080. doi:10.3748/wjg.v27.i47.8069

frontiers | Frontiers in Pharmacology

TYPE Original Research
PUBLISHED 09 December 2022
DOI 10.3389/fphar.2022.1026337



OPEN ACCESS

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SPECIALTY SECTION
This article was submitted to Gastrointestinal and Hepatic Pharmacology, a section of the journal Frontiers in Pharmacology

RECEIVED 23 August 2022
ACCEPTED 28 November 2022
PUBLISHED 09 December 2022

CITATION
Lei J, Chen B, Song M, Zhang L, Zhang X, Gao X, Li Y, Lu Y and Zuo S (2022), TKI or TKI combined with PD-1 inhibitors as second-line treatment for HCC patients after sorafenib failure.
*Front. Pharmacol.* 13:1026337.
doi: 10.3389/fphar.2022.1026337



# TKI or TKI combined with PD-1 inhibitors as second-line treatment for HCC patients after sorafenib failure

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**Background:** Tyrosine kinase inhibitors (TKI) in combination with programmed cell death-1 (PD-1) inhibitors become the potential treatment modality for patients undergoing unresectable hepatocellular carcinoma (uHCC) in the first-line setting. However, the efficacy and safety of this combination regimen in patients after sorafenib failure remains unclear.

**Methods:** Participants in this study included patients with uHCC after sorafenib failure who received TKI monotherapy (TKI group) or TKI combined with PD-1 inhibitors therapy (combination group) in our center from July 2018 to July 2021. The overall survival (OS) was used to be the primary efficacy endpoint, while progression-free survival (PFS), objective response rate (ORR), and disease control rate (DCR) were applied to be secondary endpoints. In addition, the adverse events are recorded and evaluated.

**Results:** Among the 92 patients contained in this work, 50 patients were categorized into the TKI group, while 42 patients were in the combination group. There existed no evident differences between the two groups concerning the ORR (8.0% vs. 9.5%, $p$ = 1.000). However, the DCR in the combined group was better in relative to that in the TKI group (71.4% vs. 50.0%, $p$ = 0.037). In comparison with the TKI group, it was found that the combination group presented notably better median PFS (8.1 months vs. 4.7 months, $p$ = 0.005) and median OS (21.9 months vs. 16.6 months, $p$ = 0.042). According to multivariate analysis, PFS (HR 0.5, 95% CI: 0.3–0.8, $p$ = 0.005) and OS (HR 0.5, 95% CI: 0.3–1.0, $p$ = 0.051) were improved in the combination group in relative to the TKI group after the adjustment for some risk factors. Additionally, the incidence rates of grade ≥1 adverse event in the TKI group and the combination group were 96.0% and 97.6%, respectively. The most normal adverse event in the TKI group was neutropenia ($n$ = 24,48.0%) and the combination group was hypoalbuminemia ($n$ = 23,54.8%). All of these adverse events improved after symptomatic treatment, and no new toxic events were found to occur.

**Conclusion:** TKI combined with PD-1 inhibitors showed better prognosis with manageable toxicity in uHCC patients after sorafenib failure compared with TKI monotherapy.

KEYWORDS

**hepatocellular carcinoma, tyrosine kinase inhibitor (EGFR-TKI), programmed death-1 inhibitor, second-line, sorafenib**

# Introduction

According to the latest statistics, primary liver cancer ranks the sixth most normal cancer type globally, with more than 900,000 new cases every year (Sung et al., 2021). Hepatocellular carcinoma (HCC) is found to occupy 85%–90% among all the primary liver cancers (El-Serag and Rudolph, 2007). Due to the early asymptomatic and rapid progress, most patients were diagnosed with advanced-stage disease. Patients who lost the opportunity of local therapy could only choose the best supportive treatment until the emergence of sorafenib brought them hope in 2007 (Llovet et al., 2008). The first-line treatment drugs approved by the Food and Drug Administration (FDA), from sorafenib in 2007 to lenvatinib with non-inferior effect to sorafenib in 2018, and then to atezolizumab plus bevacizumab (A + T) with excellent effect to sorafenib in 2020, have enhanced the prognosis of HCC patients and increased the selectivity of treatment schemes (Kudo et al., 2018; Cheng et al., 2022). The American Gastroenterological Association (AGA) suggests for patients with preserved liver function, A + T can improve the OS of patients with sorafenib but exclude those who are not suitable for immunotherapy and/or are at a high risk of bleeding (Su et al., 2022). Compared with sorafenib, lenvatinib has promising progression-free survival (PFS), but is more prone to hypertension and skin adverse events. The A + T regimen may become the mainstream of the first-line treatment regimen for patients undergoing HCC, but sorafenib will continue to be used to become a first-line therapy for those suffering from HCC for a long period.

As the oral small molecule multityrosine kinase inhibitor (TKI) that can hinder angiogenesis, sorafenib generates an anticancer impact through hindering vascular endothelial growth factor receptor (VEGFR) and fibroblast growth factor receptor (FGFR) (Morse et al., 2019). Although sorafenib significantly prolonged the OS of patients compared with placebo, disease control rate (DCR) was only 43% and PFS approximately 4 months, indicating that more than half of patients did not respond and patients who responded developed resistance in a short time (Llovet et al., 2008; Kudo et al., 2018). In the face of the non-response and high drug resistance rate of sorafenib, active anti-tumor treatment in the back line can benefit the survival of patients. Currently, the second-line treatment approved by FDA includes cabozantinib, regorafenib, pembrolizumab and ramucirumab (HCC patients with AFP>400 ng/ml). These second-line drugs have significantly prolonged OS in HCC patients after sorafenib failure compared with placebo, whereas the lack of head-to-head clinical data limits the level of evidence for second-line treatment options. Clinicians choose second-line treatment schemes mostly based on work experience rather than experimental evidence.

In recent years, PD-1 inhibitors have benefited a variety of cancers. Even though the use of nivolumab and pembrolizumab in HCC patients has promoted the treatment of HCC patients into the era of immunity, the curative effect is not satisfactory (Finn et al., 2020b; Yau et al., 2022). However, TKI combined with PD-1 inhibitors has become the promising treatment option. In KEYNOTE-524, pembrolizumab combined with lenvatinib significantly improved the median OS (22 months) of patients with unresectable HCC(uHCC) (Finn et al., 2020a). In RESCUE, the 18-month survival rate of HCC patients reached 58.1% by camrelizumab combination with apatinib (Xu et al., 2021). Although there are no reports of randomized controlled trials of TKI combined with PD-1 inhibitors compared with TKI monotherapy as the first-line treatment for HCC, in retrospective studies, numerous studies have revealed that the combined treatment of OS and PFS is significantly better than TKI monotherapy (Li et al., 2022; Wu et al., 2022). Nevertheless, it is not clear whether TKI combined with PD-1 inhibitors is better than TKI alone in the second-line treatment. Considering the dilemma of choosing the second-line treatment, and the significant advantages of TKI combined with PD-1 inhibitors in the first-line treatment environment, it is likely to become the best choice for the second-line treatment after sorafenib failure. Additionally, this work attempted to compare the efficacy and safety of TKI monotherapy and TKI in combination with PD-1 inhibitors in HCC patients after sorafenib failure.

# Methods

## Study design and participants

This is the retrospective research carried out in the fifth medical center of the General Hospital of the Chinese people's Liberation Army in China. From July 2018 to July 2021, HCC patients receiving TKI or TKI combined with PD-1 inhibitors as second-line treatment were included. The eligibility criteria included (1) patients diagnosed with uHCC pathologically or by two imaging techniques following the American Association for the Study of Liver Diseases (AASLD) guidelines (Marrero et al., 2018); (2) Child-Pugh class A or B; (3) an Eastern Cooperative

Oncology Group (ECOG) scale performance score of 0–1; (4) tumor progression after first-line sorafenib therapy; and (5) at least one measurable tumor lesion. Besides, the exclusion criteria contained: (1) current or a history of another malignant tumor; (2) discontinued sorafenib due to the unacceptable toxicity; and (3) missing data. The approval of this study was obtained from the Chinese registered clinical trial ethics committee, and the implementation scheme was in consistence with the declaration of Helsinki in 1975. Patients are treated according to the dosage and method of TKI or PD-1 inhibitors recommended in the relevant instructions. All included patients were divided into TKI monotherapy group (TKI group) and TKI combined with PD-1 inhibitors treatment group (combination group) using different treatment methods. Demographic characteristics (including age and gender), blood indicators (including liver function, coagulation function, routine blood and tumor markers), and characteristics were collected and evaluated at baseline.

## Endpoints and follow-up

OS was the primary endpoint of this work, which referred to the time interval from initiation of treatment to death from any reason or end of the study, whichever came the first. The secondary endpoints of this work contained progression-free survival (PFS) (determined as the time from the initial dose to the first radiologically confirmed progressive disease (PD) or death from any cause), disease control rate (DCR), and objective response rate (ORR). After treatment initiation, we recorded radiological response by dynamic computed tomography (CT) or magnetic resonance imaging (MRI) at baseline and every 8–12 weeks. The Response Evaluation Criteria in Solid Tumors (RECIST) was adopted for evaluating tumor response. According to the National Cancer Institute Common Terminology Criteria for Adverse Events version 5.0, we assessed adverse events (AEs).

## Statistical analysis

Categorical data are shown to be the frequency with proportion and explored based on Chi-square test or Fisher's exact test. With the aim of calculating the PFS and OS and plot the curve, the Kaplan-Meier method was employed. The log-rank test was adopted for comparing the two groups. A 2-tailed $p$-value ≤0.05 represented statistical significance. Cox proportional hazards models were applied, aiming to explore the correlation between the covariates and PFS or OS. Variables showing $p < 0.05$ in univariate analysis were subjected to stepwise multivariate analysis. Moreover, all data calculations were conducted by employing R language version 4.0.4 (R Foundation for Statistical Computing, Vienna, Austria).

# Results

## Patient characteristics

Totally 121 patients with unresectable HCC after failure on sorafenib were screened here from July 2018 to July 2021 in our center. Among them, we excluded 29 patients, containing 9 patients who did not receive treatment as prescribed, 7 patients who were intolerant after receiving sorafenib, 5 patients undergoing liver resection before systemic therapy, 4 patients lacking any effective follow-up, 2 patients with BCLC stage A, and 1 patient without evaluable lesions. Finally, totally 92 patients met the inclusion and exclusion criteria, including 50 in the TKI group and 42 in the combination group. The agents in TKI group included lenvatinib ($n$ = 39, 78.0%), regorafenib ($n$ = 8, 16.0%) and apatinib (n = 3, 6.0%). The main combination therapies included sorafenib plus sintilimab ($n$ = 21, 50.0%) and lenvatinib plus camrelizumab ($n$ = 6, 14.2%) (Supplementary Figure S1). At the time of data cutoff (August 2022), the median duration of follow-up was 19 (95% CI: 16.5–21.4) months. The patients were mainly male (n = 79, 85.9%). The BCLC stage of 80 (87.0%) patients was stage C at the time of enrollment. The etiology was mainly HBV(n = 85,92.4%), and there were 55 patients (60.0%) with extrahepatic metastasis. No significant difference was found in all baseline data between the sorafenib TKI group and the combination group (Table 1).

## Efficacy

All patients had at least one follow-up image for radiological tumor response assessment (Table 2). It was found that the ORR rates of the combination group and TKI group were 8.0% and 9.5%, separately. The DCR of the combination group was better than TKI group (71.4% vs. 50.0%, $p$ = 0.037). Efficacy in the combination group was statistically better than that in TKI group in terms of OS [median (95% CI): 21.9 (NE-NE) vs. 16.6 (10.2–23.0) months, $p$ = 0.042] and PFS [median (95% CI):8.1 (5.9–10.3) vs. 4.7 (3.2–6.2) months, $p$ = 0.006] (Figure 1).

## Subgroup analysis

The patients in the TKI group were classified into lenvatinib ($n$ = 39) and other TKI($n$ = 11) groups. The median OS was 14.7 (95% CI: 6.7–22.7) months in lenvatinib group, while the 13 months survival rate in the control group was 18.4%(95% CI: 3.2–33.6) ($p$ = 0.291)(Figure 2A).The median PFS was 5.5 (95% CI: 3.8–7.2) months in lenvatinib group, while the control group was 3.5 (95% CI: 2.2–4.7) months ($p$ = 0.174) (Figure 2B). In the subgroup analysis in the combination group, there existed no obvious difference in median PFS (8.3 vs. 7.1 months, $p$ = 0.364) and median OS (NE vs. 21.9 months,

TABLE 1 Baseline patient characteristics.

| | TKI group (n = 50), n (%) | Combination group (n = 42), n (%) | P |
|---|---|---|---|
| Age (mean ± SD) | 53.4 ± 8.73 | 54.9 ± 8.51 | 0.411 |
| Gender | | | 0.734 |
| Female | 6 (12.0) | 7 (16.7) | |
| Male | 44 (88.0) | 35 (83.3) | |
| Diabetes | 13 (26.0) | 7 (16.7) | 0.408 |
| Hypertension | 16 (32.0) | 8 (19.0) | 0.242 |
| Smoking | 24 (48.0) | 19 (45.2) | 0.956 |
| Alcohol Consumption | 17 (34.0) | 18 (42.9) | 0.512 |
| Chronic Liver Disease | | | 0.698 |
| HBV | 47 (94.0) | 38 (90.5) | |
| HCV | 3 (6.0) | 4 (9.5) | |
| Maximal Diameter | | | 1.000 |
| <5 cm | 22 (44.0) | 19 (45.2) | |
| ≥5 cm | 28 (56.0) | 23 (54.8) | |
| PS score | | | 1.000 |
| 0 | 36 (72.0) | 30 (71.4) | |
| 1 | 14 (28.0) | 12 (28.6) | |
| BCLC | | | 1.000 |
| B | 7 (14.0) | 5 (11.9) | |
| C | 43 (86.0) | 37 (88.1) | |
| Child Pugh | | | 0.719 |
| A | 35 (70.0) | 27 (64.3) | |
| B (total) | 15 (30.0) | 15 (35.7) | |
| B 7 | 11 (22.0) | 10 (23.8) | |
| B 8 | 4 (8.0) | 5 (11.9) | |
| Macrovascular tumor thrombosis | 26 (52.0) | 24 (57.1) | 0.777 |
| Extrahepatic metastasis | 27 (54.0) | 28 (66.7) | 0.307 |
| AFP | | | 0.098 |
| <200 | 30 (60.0) | 17 (40.5) | |
| ≥200 | 20 (40.0) | 25 (59.5) | |

HBV, hepatitis B virus; HCV, hepatitis C virus; PS, performance status; BCLC, barcelona clinic liver cancer; AFP, alpha-fetoprotein.

TABLE 2 Tumor response.

| | TKI group (n = 50), n (%) | Combination group (n = 42), n (%) | P |
|---|---|---|---|
| PR | 4 (8.0) | 4 (9.5) | |
| SD | 21 (42.0) | 26 (61.9) | |
| PD | 25 (50.0) | 12 (28.5) | |
| ORR | 4 (8.0) | 4 (9.5) | 1.000 |
| DCR | 25 (50.0) | 30 (71.4) | 0.037 |
| mPFS (months) | 4.7 | 8.1 | 0.005 |
| mOS (months) | 16.6 | 21.9 | 0.042 |

PR, partial response; SD, stable disease; PD, progressive disease; ORR, objective response rate; DCR, disease control rate; mPFS, median progression free survival; mOS, median overall survival.

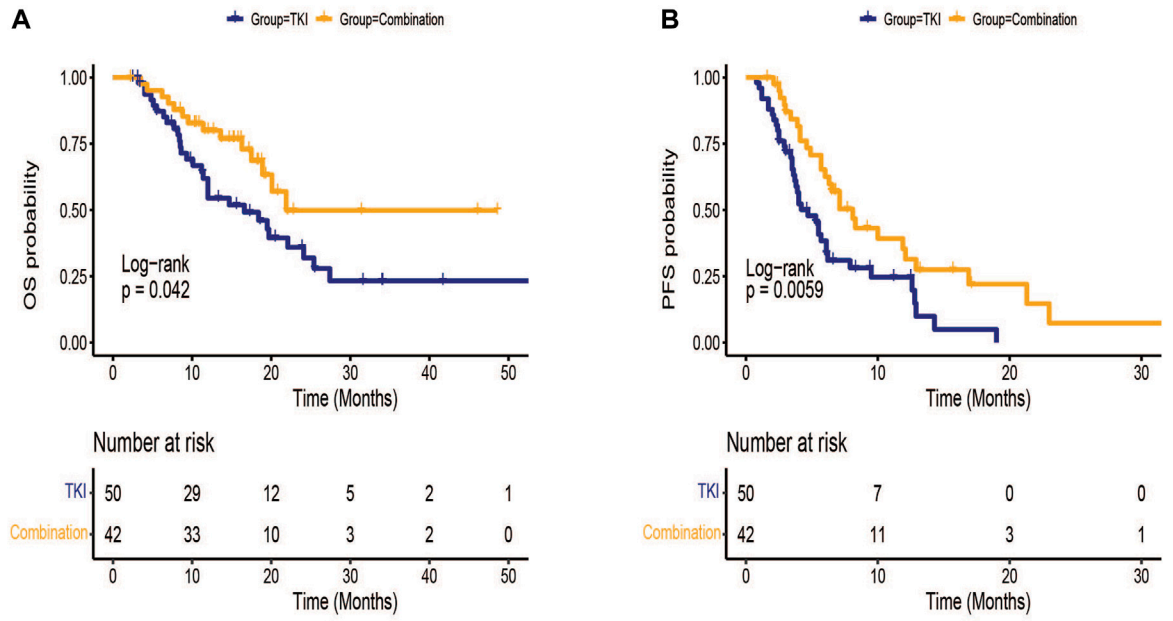


FIGURE 1
Kaplan-Meier survival curves of treatment outcome including **(A)** overall survival (OS) and **(B)** progression-free survival(PFS) between TKI group and combination group.

$p$ = 0.657) between sorafenib in combination with PD-1 inhibitors and lenvatinib in combination with PD-1 inhibitors (Figures 2C,D).

## Factors influencing efficacy

Table 3 shows the factors associated with the patient's PFS and OS. In univariate analysis, TKI monotherapy, ECOG-PS score 1, and maximum tumor diameter greater than 5 cm were independently related to a shortened PFS, while TKI monotherapy and ECOG-PS score 1 were independently associated for shortened OS. In multivariate analysis, the independently correlated with a shortened PFS included, ECOG-PS score 1 (HR 1.8, 95% CI: 1.1–3.0, $p$ = 0.027) and tumor diameter greater than 5 cm (HR 1.8, 95% CI: 1.1–2.9, $p$ = 0.028), whereas the independently associated with a shortened OS was only ECOG-PS score 1 (HR 1.9, 95% CI: 1.0–3.6, $p$ = 0.021). The combination group had better PFS (HR 0.5, 95% CI: 0.3–0.8, $p$ = 0.005) and prolonged OS (HR 0.5, 95% CI: 0.3–1.0, p = 0.051) compared to the TKI group.

## Safety

The incidence rates of grade ≥1 adverse event in the TKI group and the combination group were 96.0% and 97.6%, respectively. Obviously, the most common adverse event in the TKI group was neutropenia ($n$ = 24,48.0%) and hypoalbuminemia ($n$ = 23, 54.8%) in the combination group. In addition, the common grade 3–4 adverse events in the TKI group were leukopenia ($n$ = 6, 12.0%), thrombocytopenia ($n$ = 6, 12.0%), hypertension ($n$ = 3, 6.0%), and lymphopenia ($n$ = 3, 6.0%). The common grade 3–4 adverse events in the combined group included lymphopenia ($n$ = 9, 21.4%), leukopenia ($n$ = 3, 9.5%), hypertension ($n$ = 2, 4.7%), and thrombocytopenia ($n$ = 2, 4.7%). In the TKI group, the agent dose was decreased in 1 case due to grade 3 hypertension. In the combination group, two patients discontinued immunotherapy, including 1 with immune-related pneumonitis and 1 with immune-related myocarditis. No patients died due to adverse events (Table 4).

# Discussion

It is acknowledged that this is the first retrospective cohort study comparing treatment response and adverse events between TKI alone and TKI combined with PD-1 inhibitors as a second-line for uHCC. Our findings showed that combination therapy may improve DCR, PFS, and OS in patients in comparison with TKI monotherapy. There existed no statistically significant difference in adverse events between the two groups.

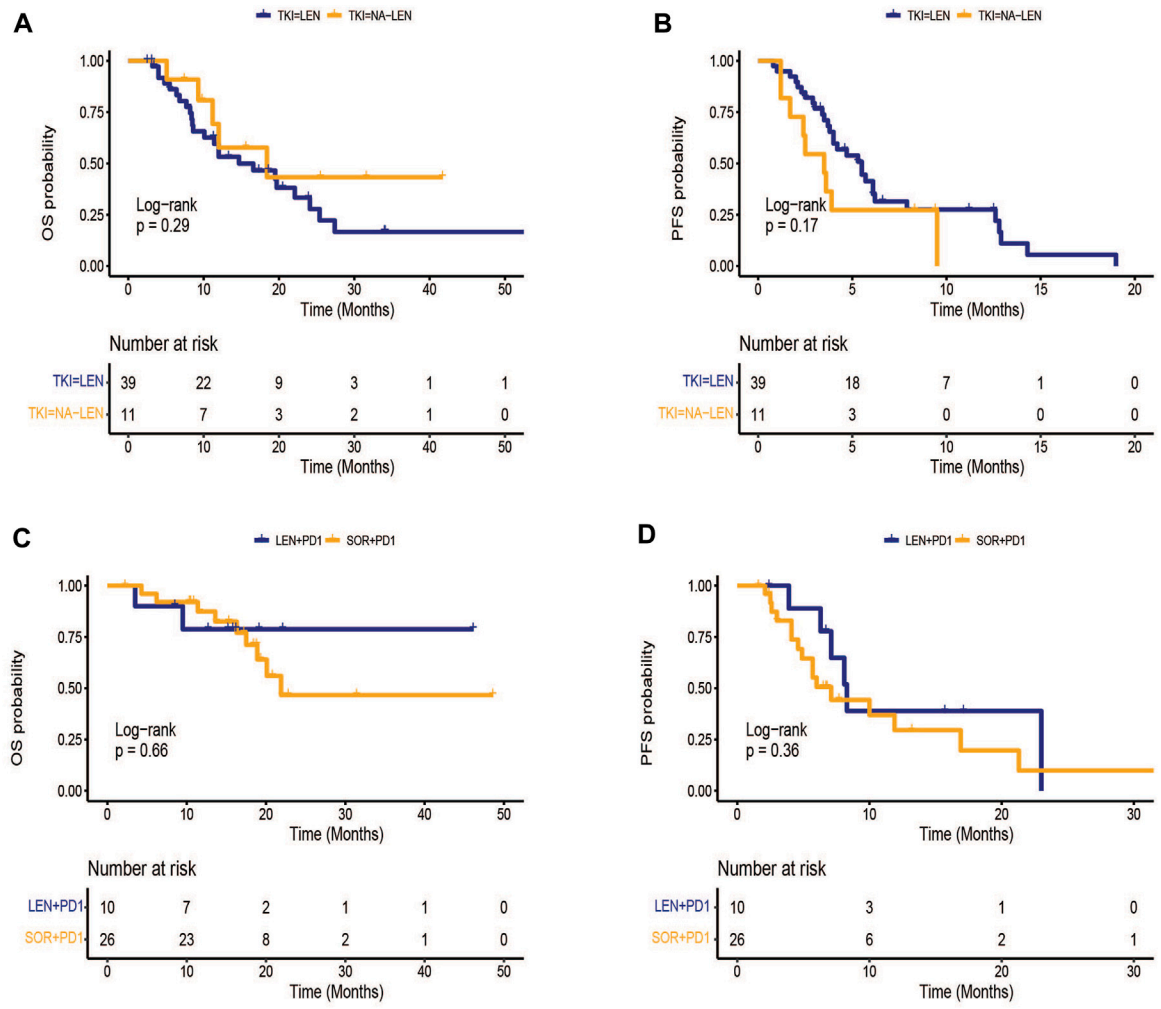


FIGURE 2
Kaplan-Meier survival curves of treatment outcome including **(A)** overall survival (OS), **(B)** progression-free survival(PFS) between lenvatinib and other TKI groups, **(C)** OS, **(D)** PFS between sorafenib plus PD-1 inhibitors and lenvatinib plus PD-1 inhibitors.

In the IMbrave 150 study, the PFS (6.9 months vs. 4.3 months, $p < 0.001$) and OS (19.2 months vs. 13.4 months, $p < 0.001$) of the A + T regimen were significantly prolonged compared with sorafenib monotherapy, and thus the regimen was recommended by the FDA as the standard first-line treatment regimen for uHCC patients (Cheng et al., 2022). The success of this combination therapy has brought novel hope to patients, and the synergistic effect of anti-vascular drugs combined with immune checkpoint inhibitors has already become the focus of patients and doctors. In prospective studies, TKI combined with PD-1 inhibitors (including pembrolizumab plus lenvatinib and camrelizumab plus apatinib) had a promising OS [(Finn et al., 2020a; Xu et al., 2021)]. In the real world, TKI combined with PD-1 inhibitors therapy has obviously prolonged OS in comparison with TKI monotherapy, including lenvatinib plus nivolumab vs. lenvatinib monotherapy (22.9 months vs. 10.3 months, $p = 0.01$) (Wu et al., 2022), lenvatinib plus camrelizumab vs. lenvatinib monotherapy (not reached vs. 13.9 months, $p = 0.02$) (Li et al., 2022), and lenvatinib plus sintilimab vs. lenvatinib monotherapy (21.7 months vs. 12.8 months, $p = 0.01$) (Zhao et al., 2022). Based on the above study, lenvatinib combined with PD-1 inhibitors had a significantly prolonged OS in first-line treatment of uHCC compared with lenvatinib monotherapy. The above studies showed that lenvatinib combined with PD-1 inhibitors significantly prolonged OS in first-line treatment of uHCC compared with lenvatinib monotherapy. In this work, although the TKI of the combination regimen was mainly sorafenib (61.9%) and was used in the second-line treatment of uHCC, the combination regimen also had a better prognosis than TKI monotherapy.

TABLE 3 Analysis of prognostic risk factors.

| | Progression-free survival | | | Overall survival | | |
|---|---|---|---|---|---|---|
| | HR | 95% CI | P | HR | 95% CI | P |
| Univariate analysis | | | | | | |
| Age>60, yeares | 0.9 | 0.4–1.9 | 0.700 | 0.4 | 0.1–1.4 | 0.160 |
| TKI group | 0.5 | 0.3–0.8 | 0.007 | 0.5 | 0.3–1.0 | 0.045 |
| Male sex | 1.2 | 0.6–2.3 | 0.650 | 1.3 | 0.5–3.0 | 0.600 |
| Diabetes | 1.3 | 0.7–2.5 | 0.370 | 1.0 | 0.5–2.0 | 0.930 |
| Hypertension | 1.3 | 0.8–2.3 | 0.310 | 1.9 | 0.8–4.5 | 0.150 |
| Smoking | 1.0 | 0.6–1.7 | 0.900 | 1.1 | 0.6–1.9 | 0.860 |
| Alcohol-Consumption | 1.1 | 0.7–1.8 | 0.650 | 0.9 | 0.5–1.7 | 0.810 |
| HCV | 1.1 | 0.4–2.7 | 0.850 | 1.3 | 0.5–3.5 | 0.670 |
| PS score 1 | 1.9 | 1.1–3.2 | 0.015 | 2.1 | 1.1–3.9 | 0.018 |
| Largest tumor size ≥5 cm | 1.7 | 1.0–2.8 | 0.040 | 1.2 | 0.7–2.3 | 0.480 |
| BCLC (C) | 1.1 | 0.6–2.3 | 0.710 | 0.9 | 0.4–2.1 | 0.800 |
| Child-Pugh | 1.6 | 0.9–2.6 | 0.100 | 1.0 | 0.5–1.9 | 0.940 |
| Macrovascular tumor thrombosis | 1.3 | 0.8–2.2 | 0.270 | 1.3 | 0.7–2.3 | 0.440 |
| Extrahepatic metastasis | 0.8 | 0.5–1.4 | 0.450 | 0.8 | 0.5–1.5 | 0.570 |
| AFP>=200 ng/ml | 1.1 | 0.7–1.7 | 0.820 | 0.9 | 0.5–1.5 | 0.590 |
| Multivariate analysis | | | | | | |
| Combination group | 0.5 | 0.3–0.8 | 0.005 | 0.5 | 0.3–1.0 | 0.051 |
| PS score 1 | 1.8 | 1.1–3.0 | 0.027 | 2.1 | 1.1–3.8 | 0.021 |
| Largest tumor size ≥5 cm | 1.8 | 1.1–2.9 | 0.028 | | | |

HR, hazard ratio; CI, confidence interval; HCV, hepatitis C virus; PS, performance status; BCLC, barcelona clinic liver cancer; AFP, alpha-fetoprotein.

Sorafenib significantly prolongs OS compared to placebo and is widely used worldwide as first-line therapy in uHCC patients (Llovet et al., 2008). Unfortunately, a large number of HCC patients show a poor response to sorafenib or exhibit resistance to sorafenib treatment within 6 months (Chen et al., 2015). Continuing systemic therapy after sorafenib failure is the most effective way to prolong OS. In RESORCE, in patients undergoing HCC who failed sorafenib, continued regorafenib treatment significantly prolonged OS compared with placebo (10.6 months vs. 7.8 months, $p < 0.001$). The median time to death remained longer in the regorafenib group when survival was evaluated from prior sorafenib (vs. placebo, 26.0 months vs. 19.2 months) (Finn et al., 2018). The benefit of regorafenib for patients after failure of sorafenib was further confirmed in several retrospective clinical studies (Granito et al., 2021a). Recently, many second-line treatment studies have been carried out for HCC patients after sorafenib failure. FDA-approved second-line therapy-targeted drugs that have shown survival benefits in phase 3 clinical trials, including regorafenib (mOS, 10.6 months) (Finn et al., 2018), cabozantinib (mOS, 10.2 months) (Abou-Alfa et al., 2018) and ramucirumab (mOS, 8.5 months) (Zhu et al., 2019). Approved second-line immune monotherapy include nivolumab (mOS, 15.6 months) (El-Khoueiry et al., 2018) and pembrolizumab (mOS, 13.8 months) (Finn et al., 2020b). Additionally, the combination regimen nivolumab plus ipilimumab has not completed a phase 3 clinical trial, but has received FDA accelerated approval in a second-line setting due to long OS (mOS, 22.8 months) (Yau et al., 2020). Furthermore, second-line combination therapy options that are expected to be approved are durvalumab plus tremelimumab (mOS, 18.7 months) (Kelley et al., 2021) and camrelizumab plus apatinib (18 months OS rates, 56.5%) (Xu et al., 2021). Moreover, many second-line drugs that have completed phase 2 clinical trials or have been approved in some countries are booming, including apatinib (mOS, 8.7 months) (Qin et al., 2021), tislelizumab (mOS, 12.4 months) (Ducreux et al., 2021) and camrelizumab (mOS, 13.8 months) (Qin et al., 2020).

Faced with so many second-line treatment options, how to determine the best treatment has become the most perplexing problem. To determine the best second-line treatment regimen, we performed the analysis from different perspectives. First, based on the prospective second-line studies, the combination therapy regimen has a better OS than the monotherapy (targeted therapy or immunotherapy). Nevertheless, such conclusions need to be cautious, because some of the above studies have only completed the phase 2 clinical trials, even if phase 3 clinical trials have been completed but only use placebo as a control. Second, in the real world, controlled trials of second-line drugs

TABLE 4 Treatment-related adverse events (TRAEs).

| Adverse event | TKI group (n = 50) | | Combination group (n = 42) | |
|---|---|---|---|---|
| | Grade 1–2 | Grade 3–4 | Grade 1–2 | Grade 3–4 |
| Any treatment-related adverse event, n (%) | 48 (96.0) | 15 (30.0) | 41 (97.6) | 16 (38.0) |
| Diarrhea, n (%) | 12 (24.0) | 0 | 7 (16.7) | 1 (2.3) |
| Fatigue, n (%) | 11 (22.0) | 0 | 6 (14.2) | 0 |
| Hand and foot syndrome, n (%) | 6 (12.0) | 2 (4.0) | 4 (9.5) | 1 (2.3) |
| Hypertension, n (%) | 4 (8.0) | 3 (6.0) | 5 (11.8) | 2 (4.7) |
| Decreased appetite, n (%) | 5 (10.0) | 0 | 3 (7.1) | 0 |
| Proteinuria, n (%) | 5 (10.0) | 0 | 1 (2.3) | 0 |
| Hypothyroidism, n (%) | 4 (8.0) | 0 | 1 (2.3) | 0 |
| Rash, n (%) | 2 (4.0) | 0 | 3 (7.0) | 0 |
| Immune-related pneumonia, n (%) | 0 | 0 | 1 (2.3) | 0 |
| Myocarditis, n (%) | 0 | 0 | 1 (2.3) | 0 |
| Laboratory test, n (%) | | | | |
| Neutropenia, n (%) | 24 (48.0) | 1 (2.0) | 11 (26.2) | 1 (2.3) |
| Leukopenia, n (%) | 21 (42.0) | 6 (12.0) | 13 (30.8) | 3 (9.5) |
| Hypoalbuminemia, n (%) | 21 (42.0) | 0 | 23 (54.8) | 0 |
| Fibrinogen decreased, n (%) | 19 (38.0) | 0 | 13 (30.8) | 0 |
| Lymphopenia, n (%) | 16 (32.0) | 3 (6.0) | 8 (19.0) | 9 (21.4) |
| Alanine aminotransferase increased, n (%) | 16 (32.0) | 1 (2.0) | 5 (11.9) | 0 |
| Thrombocytopenia, n (%) | 15 (30.0) | 6 (12.0) | 12 (28.5) | 2 (4.7) |
| Blood lactate dehydrogenase increased, n (%) | 13 (26.0) | 0 | 10 (23.8) | 0 |
| Aspartate aminotransferase increased, n (%) | 12 (24.0) | 1 (2.0) | 7 (16.7) | 0 |
| Hypocalcemia, n (%) | 10 (20.0) | 0 | 13 (31.0) | 0 |
| Hypokalemia, n (%) | 10 (20.0) | 0 | 14 (33.4) | 0 |
| Anemia, n (%) | 8 (16.0) | 1 (2.0) | 12 (28.5) | 0 |
| Hypophosphatemia, n (%) | 8 (16.0) | 0 | 13 (31.0) | 0 |
| Hyperuricemia, n (%) | 5 (10.0) | 0 | 4 (9.5) | 0 |
| Serum amylase increased, n (%) | 4 (8.0) | 0 | 4 (9.6) | 0 |
| Creatinine increased, n (%) | 0 | 0 | 3 (7.1) | 0 |

only compared single agents and did not screen for superiority, including regorafenib *versus* nivolumab (Choi et al., 2020), regorafenib *versus* cabozantinib (Casadei-Gardini et al., 2021) and cabozantinib *versus* ramucirumab (Trojan et al., 2021). Our results suggest that there is a marginal difference in PFS with lenvatinib compared with other TKI agents (5.5 vs. 3.5 months, $p = 0.147$). Previously, lenvatinib is superior to sorafenib of PFS in both prospective and retrospective studies (Kudo et al., 2018; Kuo et al., 2021), and thus it may be preferentially recommended in patients who cannot use immunotherapy after sorafenib failure. Certainly, for patients who can use immunotherapy, TKI combined with PD-1 inhibitors as a second-line regimen is a good option in line with our results. Third, the same treatment may exert different effects in different countries or regions. The primary risk factor for non-Japanese Asian patients is HBV, while European and American patients are HCV (El-Serag, 2012). The median OS of HCC patients treated with sorafenib was 10.7 months in Europe, Australasia and the United States, and 6.5 months in China, Taiwan, and South Korea (Llovet et al., 2008; Cheng et al., 2009). Due to the differences in regions and etiologies, although apatinib has been approved to be the second-line treatment in China, the efficacy of this regimen in other countries needs further investigation since the phase 3 clinical trial only included Chinese patients (Qin et al., 2021). Fourth, the current studies on second-line therapy choices for HCC patients are all conducted with sorafenib as a first-line treatment. Intolerance or disease progression due to sorafenib is related to response to second-line therapy. Ramucirumab and pembrolizumab were effective for patients with disease progression after sorafenib treatment, but not for the intolerant to sorafenib (Zhu et al., 2019; Finn et al., 2020b). In RESORCE, patients who were intolerant to

sorafenib were excluded from the enrolled patients receiving second-line regorafenib excluded. However, cabozantinib can bring benefits after sorafenib treatment in patients with disease progression or intolerance, and thus it is the only second-line TKI recommended by the AGA for use in patients with sorafenib intolerance (Kelley et al., 2020; Su et al., 2022). Our study also excluded sorafenib-intolerant patients. Therefore, the efficacy of TKI combined with PD-1 inhibitors in sorafenib-intolerant patients in the second-line setting needs to be further explored in follow-up studies.

A comprehensive analysis of the above-mentioned second-line treatment decision-making perspectives, combined with our findings, shows that there is potential value in recommending combination therapy after sorafenib failure. (1) At present, the commonly used second-line single drugs are TKI drugs, and thus there may be cross-resistance with sorafenib which can greatly limit the survival of patients. (2) Commonly used TKI drugs all exert the targeted therapeutic effect of VEGFR, which can not only regulate tumor blood vessels, but also serve as an effective immunomodulatory molecule, affecting TAM, MDSC, Treg cells and effector T cells (Fukumura et al., 2018). Nevertheless, PD-1 inhibitors can restore effector $CD8^+$ T cell function by blocking extensive dephosphorylation between PD-L1 and PD-1, which can impair or abolish the immunosuppressive effects caused by Treg cells and ultimately inhibit tumor growth (Ahn et al., 2018; Granito et al., 2021b). Multiple mouse experiments have demonstrated that TKI combined with PD-1 inhibitors combination therapy can achieve the synergistic effect (Sprinzl et al., 2015; Torrens et al., 2021). (3) Multiple studies have revealed that the toxicity profile and tolerance were similar between TKI monotherapy and combination regimens (Li et al., 2022; Wu et al., 2022). (4) There are many combinations of TKI combined with PD-1 inhibitors, which can avoid the limitations of a certain drug and increase the practicality of the treatment plan. Our subgroup analysis proved that there existed no obvious difference in OS and PFS between sorafeinib combined with PD-1 inhibitors and lenvatinib combined with PD-1 inhibitors, which could also increase the possibility that different combinations may benefit from. However, the mechanism of lenvatinib and sorafenib combined with PD-1 inhibitors is different, the former can specifically reduce the abundance of tumor Treg cells (Torrens et al., 2021), while the latter has the effect of directly inhibiting the activation of M2 macrophages (Sprinzl et al., 2015). The effect of this combination treatment is promising. However, follow-up large-sample and prospective studies need to be performed to explore what kind of combination is more effective and what kind of situation is used.

Several limitations have to be mentioned in this study. First, this study was designed as a retrospective one with the small sample size, which could generate information bias and selection bias. Moreover, we explored multiple second-line TKI or PD-1 inhibitors. The clinical efficacy of specific regimens must be explored in future clinical trials. Third, our study excluded patients with sorafenib intolerance. Thus, the efficacy of TKI in combination with PD-1 inhibitors was not available in these patients.

## Conclusion

To conclude, TKI combined with PD-1 inhibitors may benefit more than TKI monotherapy in HCC patients after sorafenib failure. Prospective studies with large samples are required to explore and clarify specific treatment options for patients.

## Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding authors.

## Ethics statement

The studies involving human participants were reviewed and approved by China registered clinical trial ethics committee before the study (Approval Number: ChiECRCT20210348). Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

## Author contributions

JL, BC, and MS conducted the experiment and executed most of the data processing and analysis and wrote the manuscript. LZ, XZ, XG, and YL conducted the experiments and analyzed the data. YL and SZ participated in the designing of the experiments and data analysis and guided and supervised the work. All authors read and approved the submitted version.

## Funding

This study was supported by grants from Science Technology and Innovation Committee of Shenzhen Municipality (2019 N002) of China and Special Fund for Outstanding Young Scientific and Technological Talents of Guizhou Province (Talents of Guizhou Kehe platform [2019]5647).

## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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## Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphar.2022.1026337/full#supplementary-material

## References

Abou-Alfa, G. K., Meyer, T., Cheng, A-L., El-Khoueiry, A. B., Rimassa, L., Ryoo, B-Y., et al. (2018). Cabozantinib in patients with advanced and progressing hepatocellular carcinoma. *N. Engl. J. Med.* 379 (1), 54–63. doi:10.1056/NEJMoa1717002

Ahn, E., Araki, K., Hashimoto, M., Li, W., Riley, J. L., Cheung, J., et al. (2018). Role of PD-1 during effector CD8 T cell differentiation. *Proc. Natl. Acad. Sci. U. S. A.* 115 (18), 4749–4754. doi:10.1073/pnas.1718217115

Casadei-Gardini, A., Rimassa, L., Rimini, M., Yoo, C., Ryoo, B-Y., Lonardi, S., et al. (2021). Regorafenib versus cabozantinib as second-line treatment after sorafenib for unresectable hepatocellular carcinoma: Matching-adjusted indirect comparison analysis. *J. Cancer Res. Clin. Oncol.* 147 (12), 3665–3671. doi:10.1007/s00432-021-03602-w

Chen, J., Jin, R., Zhao, J., Liu, J., Ying, H., Yan, H., et al. (2015). Potential molecular, cellular and microenvironmental mechanism of sorafenib resistance in hepatocellular carcinoma. *Cancer Lett.* 367 (1), 1–11. doi:10.1016/j.canlet.2015.06.019

Cheng, A-L., Kang, Y-K., Chen, Z., Tsao, C-J., Qin, S., Kim, J. S., et al. (2009). Efficacy and safety of sorafenib in patients in the asia-pacific region with advanced hepatocellular carcinoma: A phase III randomised, double-blind, placebo-controlled trial. *Lancet. Oncol.* 10 (1), 25–34. doi:10.1016/S1470-2045(08)70285-7

Cheng, A-L., Qin, S., Ikeda, M., Galle, P. R., Ducreux, M., Kim, T-Y., et al. (2022). Updated efficacy and safety data from IMbrave150: Atezolizumab plus bevacizumab vs. sorafenib for unresectable hepatocellular carcinoma. *J. Hepatol.* 76 (4), 862–873. doi:10.1016/j.jhep.2021.11.030

Choi, W-M., Choi, J., Lee, D., Shim, J. H., Lim, Y-S., Lee, H. C., et al. (2020). Regorafenib versus nivolumab after sorafenib failure: Real-world data in patients with hepatocellular carcinoma. *Hepatol. Commun.* 4 (7), 1073–1086. doi:10.1002/hep4.1523

Ducreux, M., Abou-Alfa, G., Ren, Z., Edeline, J., Li, Z., Assenat, E., et al. (2021). O-1 Results from a global phase 2 study of tislelizumab, an investigational PD-1 antibody, in patients with unresectable hepatocellular carcinoma. *Ann. Oncol.* 32, S217. doi:10.1016/j.annonc.2021.05.005

El-Khoueiry, A. B., Melero, I., Yau, T. C., Crocenzi, T. S., Kudo, M., Hsu, C., et al. (2018). Impact of antitumor activity on survival outcomes, and nonconventional benefit, with nivolumab (NIVO) in patients with advanced hepatocellular carcinoma (aHCC): Subanalyses of CheckMate-040. *J. Clin. Oncol.* 36, 475. doi:10.1200/jco.2018.36.4_suppl.475

El-Serag, H. B. (2012). Epidemiology of viral hepatitis and hepatocellular carcinoma. *Gastroenterology* 142 (6), 1264–1273. doi:10.1053/j.gastro.2011.12.061

El-Serag, H. B., and Rudolph, K. L. (2007). Hepatocellular carcinoma: Epidemiology and molecular carcinogenesis. *Gastroenterology* 132 (7), 2557–2576. doi:10.1053/j.gastro.2007.04.061

Finn, R. S., Ikeda, M., Zhu, A. X., Sung, M. W., Baron, A. D., Kudo, M., et al. (2020). Phase ib study of lenvatinib plus pembrolizumab in patients with unresectable hepatocellular carcinoma. *J. Clin. Oncol.* 38 (26), 2960–2970. doi:10.1200/JCO.20.00808

Finn, R. S., Merle, P., Granito, A., Huang, Y-H., Bodoky, G., Pracht, M., et al. (2018). Outcomes of sequential treatment with sorafenib followed by regorafenib for HCC: Additional analyses from the phase III RESORCE trial. *J. Hepatol.* 69 (2), 353–358. doi:10.1016/j.jhep.2018.04.010

Finn, R. S., Ryoo, B-Y., Merle, P., Kudo, M., Bouattour, M., Lim, H. Y., et al. (2020). Pembrolizumab as second-line therapy in patients with advanced hepatocellular carcinoma in KEYNOTE-240: A randomized, double-blind, phase III trial. *J. Clin. Oncol.* 38 (3), 193–202. doi:10.1200/JCO.19.01307

Fukumura, D., Kloepper, J., Amoozgar, Z., Duda, D. G., and Jain, R. K. (2018). Enhancing cancer immunotherapy using antiangiogenics: Opportunities and challenges. *Nat. Rev. Clin. Oncol.* 15 (5), 325–340. doi:10.1038/nrclinonc.2018.29

Granito, A., Forgione, A., Marinelli, S., Renzulli, M., Ielasi, L., Sansone, V., et al. (2021). Experience with regorafenib in the treatment of hepatocellular carcinoma. *Ther. Adv. Gastroenterol.* 14, 17562848211016959. doi:10.1177/17562848211016959

Granito, A., Muratori, L., Lalanne, C., Quarneti, C., Ferri, S., Guidi, M., et al. (2021). Hepatocellular carcinoma in viral and autoimmune liver diseases: Role of CD4+ CD25+ Foxp3+ regulatory T cells in the immune microenvironment. *World J. Gastroenterol.* 27 (22), 2994–3009. doi:10.3748/wjg.v27.i22.2994

Kelley, R. K., Ryoo, B-Y., Merle, P., Park, J-W., Bolondi, L., Chan, S. L., et al. (2020). Second-line cabozantinib after sorafenib treatment for advanced hepatocellular carcinoma: A subgroup analysis of the phase 3 CELESTIAL trial. *ESMO Open* 5 (4), e000714. doi:10.1136/esmoopen-2020-000714

Kelley, R. K., Sangro, B., Harris, W., Ikeda, M., Okusaka, T., Kang, Y-K., et al. (2021). Safety, efficacy, and pharmacodynamics of tremelimumab plus durvalumab for patients with unresectable hepatocellular carcinoma: Randomized expansion of a phase I/II study. *J. Clin. Oncol.* 39 (27), 2991–3001. doi:10.1200/JCO.20.03555

Kudo, M., Finn, R. S., Qin, S., Han, K-H., Ikeda, K., Piscaglia, F., et al. (2018). Lenvatinib versus sorafenib in first-line treatment of patients with unresectable hepatocellular carcinoma: A randomised phase 3 non-inferiority trial. *Lancet* 391 (10126), 1163–1173. doi:10.1016/S0140-6736(18)30207-1

Kuo, Y-H., Lu, S-N., Chen, Y-Y., Kee, K-M., Yen, Y-H., Hung, C-H., et al. (2021). Corrigendum: Real-World lenvatinib *versus* sorafenib in patients with advanced hepatocellular carcinoma: A propensity score matching analysis. *Front. Oncol.* 11, 823960. doi:10.3389/fonc.2021.823960

Li, Q., Cao, M., Yuan, G., Cheng, X., Zang, M., Chen, M., et al. (2022). Lenvatinib plus camrelizumab vs. Lenvatinib monotherapy as first-line treatment for unresectable hepatocellular carcinoma: A multicenter retrospective cohort study. *Front. Oncol.* 12, 809709. doi:10.3389/fonc.2022.809709

Llovet, J. M., Ricci, S., Mazzaferro, V., Hilgard, P., Gane, E., Blanc, J-F., et al. (2008). Sorafenib in advanced hepatocellular carcinoma. *N. Engl. J. Med.* 359 (4), 378–390. doi:10.1056/NEJMoa0708857

Marrero, J. A., Kulik, L. M., Sirlin, C. B., Zhu, A. X., Finn, R. S., Abecassis, M. M., et al. (2018). Diagnosis, staging, and management of hepatocellular carcinoma: 2018 practice guidance by the American association for the study of liver diseases. *Hepatol. Baltim. Md)* 68 (2), 723–750. doi:10.1002/hep.29913

Morse, M. A., Sun, W., Kim, R., He, A. R., Abada, P. B., Mynderse, M., et al. (2019). The role of angiogenesis in hepatocellular carcinoma. *Clin. Cancer Res.* 25 (3), 912–920. doi:10.1158/1078-0432.CCR-18-1254

Qin, S., Li, Q., Gu, S., Chen, X., Lin, L., Wang, Z., et al. (2021). Apatinib as second-line or later therapy in patients with advanced hepatocellular carcinoma (AHELP): A multicentre, double-blind, randomised, placebo-controlled, phase 3 trial. *Lancet. Gastroenterol. Hepatol.* 6 (7), 559–568. doi:10.1016/S2468-1253(21)00109-6

Qin, S., Ren, Z., Meng, Z., Chen, Z., Chai, X., Xiong, J., et al. (2020). Camrelizumab in patients with previously treated advanced hepatocellular carcinoma: A multicentre, open-label, parallel-group, randomised, phase 2 trial. *Lancet. Oncol.* 21 (4), 571–580. doi:10.1016/S1470-2045(20)30011-5

Sprinzl, M. F., Puschnik, A., Schlitter, A. M., Schad, A., Ackermann, K., Esposito, I., et al. (2015). Sorafenib inhibits macrophage-induced growth of hepatoma cells by interference with insulin-like growth factor-1 secretion. *J. Hepatol.* 62 (4), 863–870. doi:10.1016/j.jhep.2014.11.011

Su, G. L., Altayar, O., O'Shea, R., Shah, R., Estfan, B., Wenzell, C., et al. (2022). AGA clinical practice guideline on systemic therapy for hepatocellular carcinoma. *Gastroenterology* 162 (3), 920–934. doi:10.1053/j.gastro.2021.12.276

Sung, H., Ferlay, J., Siegel, R. L., Laversanne, M., Soerjomataram, I., Jemal, A., et al. (2021). Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *Ca. Cancer J. Clin.* 71 (3), 209–249. doi:10.3322/caac.21660

Torrens, L., Montironi, C., Puigvehí, M., Mesropian, A., Leslie, J., Haber, P. K., et al. (2021). Immunomodulatory effects of lenvatinib plus anti-programmed cell death protein 1 in mice and rationale for patient enrichment in hepatocellular carcinoma. *Hepatol. Baltim. Md)* 74 (5), 2652–2669. doi:10.1002/hep.32023

Trojan, J., Mollon, P., Daniele, B., Marteau, F., Martín, L., Li, Y., et al. (2021). Comparative efficacy of cabozantinib and ramucirumab after sorafenib for patients with hepatocellular carcinoma and alpha-fetoprotein ≥ 400 ng/mL: A matching-adjusted indirect comparison. *Adv. Ther.* 38 (5), 2472–2490. doi:10.1007/s12325-021-01700-2

Wu, W-C., Lin, T-Y., Chen, M-H., Hung, Y-P., Liu, C-A., Lee, R-C., et al. (2022). Lenvatinib combined with nivolumab in advanced hepatocellular carcinoma-real-world experience. *Invest. New Drugs* 40 (4), 789–797. doi:10.1007/s10637-022-01248-0

Xu, J., Shen, J., Gu, S., Zhang, Y., Wu, L., Wu, J., et al. (2021). Camrelizumab in combination with apatinib in patients with advanced hepatocellular carcinoma (RESCUE): A nonrandomized, open-label, phase II trial. *Clin. Cancer Res.* 27 (4), 1003–1011. doi:10.1158/1078-0432.CCR-20-2571

Yau, T., Kang, Y-K., Kim, T-Y., El-Khoueiry, A. B., Santoro, A., Sangro, B., et al. (2020). Efficacy and safety of nivolumab plus ipilimumab in patients with advanced hepatocellular carcinoma previously treated with sorafenib: The CheckMate 040 randomized clinical trial. *JAMA Oncol.* 6 (11), e204564. doi:10.1001/jamaoncol.2020.4564

Yau, T., Park, J-W., Finn, R. S., Cheng, A-L., Mathurin, P., Edeline, J., et al. (2022). Nivolumab versus sorafenib in advanced hepatocellular carcinoma (CheckMate 459): A randomised, multicentre, open-label, phase 3 trial. *Lancet. Oncol.* 23 (1), 77–90. doi:10.1016/S1470-2045(21)00604-5

Zhao, L., Chang, N., Shi, L., Li, F., Meng, F., Xie, X., et al. (2022). Corrigendum to "lenvatinib plus sintilimab versus lenvatinib monotherapy as first-line treatment for advanced HBV-related hepatocellular carcinoma: A retrospective, real-world study" [heliyon 8 (6) (2022) e09538]. *Heliyon* 8 (6), e11404. doi:10.1016/j.heliyon.2022.e11404

Zhu, A. X., Kang, Y-K., Yen, C-J., Finn, R. S., Galle, P. R., Llovet, J. M., et al. (2019). Ramucirumab after sorafenib in patients with advanced hepatocellular carcinoma and increased α-fetoprotein concentrations (REACH-2): A randomised, double-blind, placebo-controlled, phase 3 trial. *Lancet. Oncol.* 20 (2), 282–296. doi:10.1016/S1470-2045(18)30937-9

frontiers | Frontiers in Pharmacology

TYPE Original Research
PUBLISHED 10 January 2023
DOI 10.3389/fphar.2022.1032881



OPEN ACCESS

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SPECIALTY SECTION
This article was submitted to Gastrointestinal and Hepatic Pharmacology,a section of the journal Frontiers in Pharmacology

RECEIVED 12 September 2022
ACCEPTED 30 December 2022
PUBLISHED 10 January 2023

CITATION
Li X, Wang J, Ding X, Xu Y, Yu M, Wu H, Deng N, Li W and Chen J (2023), Clinical study of lenvatinib in the treatment of hepatitis virus-related hepatocellular carcinoma and antiviral therapy. *Front. Pharmacol.* 13:1032881. doi: 10.3389/fphar.2022.1032881



# Clinical study of lenvatinib in the treatment of hepatitis virus-related hepatocellular carcinoma and antiviral therapy

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**Background:** Lenvatinib is recommended as a first-line tyrosine kinase inhibitor for advanced hepatocellular carcinoma (HCC) since 2017. The aim of this study was to compare the clinical action of lenvatinib in hepatitis B virus (HBV)-related HCC and hepatitis C virus (HCV)-related HCC.

**Methods:** A continuous cohort of advanced HCC was retrospectively enrolled. And the patients were divided into HBV-related HCC and HCV-related HCC based on previous history of hepatitis virus infection. Then propensity score matching (PSM) was conducted to compare objective response rate (ORR),disease control rate (DCR),progression-free survival (PFS),overall survival (OS) and safety between the two groups.

**Results:** A total of 203 eligible patients were included, with 72 HBV-related HCC and 36 HCV-related HCC after PSM. Both ORR (20.8% vs. 5.6%, P = .0759) and DCR (76.4% vs. 52.8%, P = .0232) were significantly higher in the HBV-related HCC than in the HCV-related HCC. Although no statistical differences in PFS (6.1 months vs. 3.3 months, P = .17) and OS (14.9 months vs. 17.7 months, P = .96) were observed between the two groups, there was a trend of difference in the PFS survival curve. On multivariate regression analysis of PFS, both HBV infection (HR, .54; 95% CI, .31–.95; P = .0332) and antiviral time >5 years (HR, .49; 95% CI, .26–.9; P = .0219) were identified as independent favorable factors, and AFP >200 ng/mL (HR, 1.88; 95% CI, 1.1–3.22; P = .0216) were found to be an independent adverse factor. In addition, compared with HCC who received the first dose of antiviral drugs less than 5 years, the patients who were administered those drugs over 5 years had a significantly favorable PFS (11.27 months vs. 3.87 months, P = .0011). Lenvatinib was well tolerated in all patients and the adverse events (AEs) were similar between the two groups.

**Conclusion:** It seemed that lenvatinib benefited more in HBV-related advanced HCC in delaying disease progression, compared to those with HCV-related advanced HCC.

KEYWORDS

**hepatocellular carcinoma, lenvatinib, hepatitis B virus, hepatitis C virus, antiviral therapy**

## Introduction

Liver cancer is the sixth most common cancer worldwide in 2020, with about 906,000 new cases and is the third leading cause of cancer death, with about 830,000 deaths (Sung et al., 2021). Hepatocellular carcinoma (HCC) accounts for 75%–85% as the main histological type (Sung et al., 2021). Viral hepatitis is a major cause of HCC, including hepatitis B virus (HBV) and hepatitis C virus (HCV) infection (Cooke et al., 2019). HBV seroprevalence has continued to decline due to HBV vaccination, and the incidence of HCC has decreased in high-risk countries such as China and the Republic of Korea (Petrick et al., 2020). While vaccine coverage is low in sub-Saharan Africa, HBV-related HCC is still more prevalent and severe (Lemoine et al., 2016). HCV infection occurs mainly in low- and middle-income countries, and although there is no vaccine to prevent HCV infection, direct acting antiviral (DAA) drugs are highly curative and well tolerated (Lanini et al., 2016). Overall, HBV and HCV infection account for 56% and 20% of the global liver cancer deaths, with a huge disease burden (Sung et al., 2021). In clinical practice and guidelines of HCC, the treatment recommendations rely on disease stage and liver function, and they remain the same whatever the reason is HBV or HCV infection.

Early HCC can be potentially curative by resection, thermal ablation, or liver transplantation, and for unresectable patients, local treatments such as trans-arterial chemoembolization (TACE), ablation and radiotherapy can improve patients' survival (Forner et al., 2018). Moreover, up to 70% patients with HCC are diagnosed at an advanced stage and systemic therapy, such as tyrosine kinase inhibitors (TKIs), is recommended as the first-line regimen (Villanueva, 2019). Sorafenib was the first TKI approved for unresectable HCC, and exploratory analyses of SHARP (Llovet et al., 2008) and Asia-Pacific regions (Cheng et al., 2009) as well as other studies (Peixoto et al., 2014) had shown that sorafenib provided a greater magnitude of benefit in HCV-positive and/or HBV-negative HCC (Bruix et al., 2017; Jackson et al., 2017). For regions with higher HBV infection rates, the benefit of sorafenib was remarkedly smaller (Peixoto et al., 2014) until the advent of another molecular targeted drug. Lenvatinib inhibits vascular endothelial growth factor (VEGF) receptor, platelet-derived growth factor (PDFG) receptor α, fibroblast growth factor (FGF) receptor, and KIT and RET proto-oncogenes (Ikeda et al., 2017). The REFLECT trial demonstrated that lenvatinib was not inferior to sorafenib in overall survival (OS) in the first-line treatment of advanced HCC, with greater improvements in secondary study endpoints such as progression-free survival (PFS), time to progression (TTP), and objective response rate (ORR) (Kudo et al., 2018). The subgroup analysis of this study also demonstrated the benefit of PFS for HBV-related HCC in the lenvatinib group over the sorafenib group (7.3 vs. 3.6 months; HR, .62; 95% CI, .50–.75; $p < .05$) (Kudo et al., 2018). A network meta-analysis showed that lenvatinib was the best mono-therapy for HBV-related advanced HCC in the first-line treatment (Park et al., 2019). Lenvatinib showed better efficacy than sorafenib in a real-world study, and this study highlighted the negative predictive role of HCV on the lenvatinib arm (Rimini et al., 2021).

However, there are no head-to-head studies between different etiologies in HCC treating by lenvatinib, and matching is not strictly performed for comparability. The aim of this study was to compare the clinical action of lenvatinib in HBV-related HCC and HCV-related HCC.

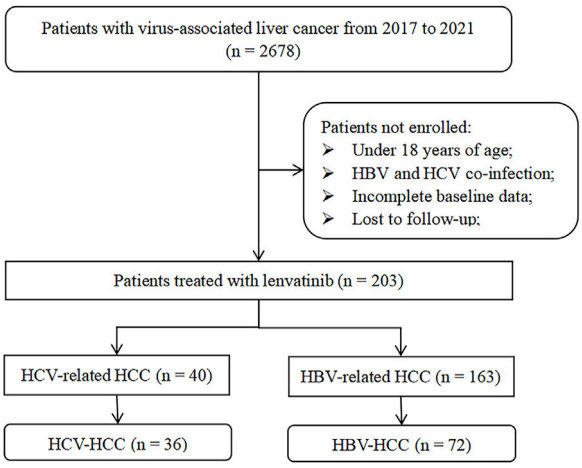


FIGURE 1
Flowchart of the study.

## Methods

### Patients

A continuous cohort of HCC who were treated with mono-lenvatinib at Beijing Ditan Hospital, Capital Medical University from October 2017 to October 2021 were retrospectively collected. Patients over 18 years with hepatitis virus-associated HCC were selected, and required to have at least one measurable lesion by modified Response Evaluation Criteria in Solid Tumors (mRECIST) (Lencioni et al., 2017). In addition, patients included had Child-Pugh grade A/B and Eastern Cooperative Oncology Group performance status (ECOG PS) ≤ 2. Patients who were not on first-line monotherapy, ie, receiving other TKIs or immunotherapy, were excluded. And we removed patients with incomplete baseline data as well as those who were lost to follow-up. Regarding the underlying etiology of hepatitis virus, HBV-related HCC included patients who were positive for HBV surface antigen (HBsAg), HBV core antibody (HBcAb) or HBV e antibody (HBeAb), while patients who were positive for HCV antibody were considered HCV-related HCC, and patients with dual HBV and HCV infection were excluded. Demographic characteristics (etiology and antiviral therapy, age, gender and ECOG PS), baseline clinical data (treatment history, imaging and laboratory parameters) and follow-up data were recorded.

The study conformed to the 1975 Declaration of Helsinki and has been approved by the Ethics Committee of Beijing Ditan Hospital, Capital Medical University. All patients provided written informed consent prior to the study.

### Treatment and assessments

Lenvatinib was administered according to the REFLECT trial (Kudo et al., 2018), and patients weighing ≥60 and <60 kg received initial oral doses of 12 and 8 mg/day, respectively. Dose reductions and

**TABLE 1 Baseline characteristics between HBV-HCC and HCV-HCC.**

| Characteristics | Overall (*n* = 108) | HBV-HCC (*n* = 72) | HCV-HCC (*n* = 36) | P |
|---|---|---|---|---|
| Age (years) | 63.5 [56.0, 69.0] | 62.0 [56.8, 69.0] | 66.0 [55.8, 69.0] | 0.6503 |
| Sex | — | — | — | — |
| Male | 97 (89.8) | 65 (90.3) | 32 (88.9) | 1 |
| Female | 11 (10.2) | 7 (9.7) | 4 (11.1) | — |
| ECOG (%) | — | — | — | 0.6216 |
| PS 0 | 53 (49.1) | 34 (47.2) | 19 (52.8) | — |
| PS 1 | 45 (41.7) | 30 (41.7) | 15 (41.7) | — |
| PS 2 | 10 (9.3) | 8 (11.1) | 2 (5.6) | — |
| Antiviral time (%) | — | — | — | 0.9388 |
| ≤5 years | 79 (73.1) | 52 (72.2) | 27 (75.0) | — |
| >5 years | 29 (26.9) | 20 (27.8) | 9 (25.0) | — |
| Cirrhosis (%) | 84 (77.8) | 55 (76.4) | 29 (80.6) | 0.8061 |
| Previous surgery (%) | 16 (14.8) | 8 (11.1) | 8 (22.2) | 0.2131 |
| Previous TACE (%) | 96 (88.9) | 65 (90.3) | 31 (86.1) | 0.7454 |
| Previous ablation (%) | 49 (45.4) | 32 (44.4) | 17 (47.2) | 0.9455 |
| Number (%) | — | — | — | 1 |
| ≤3 | 53 (49.1) | 35 (48.6) | 18 (50.0) | — |
| >3 | 55 (50.9) | 37 (51.4) | 18 (50.0) | — |
| Size (%) | — | — | — | 0.4504 |
| ≤5 cm | 61 (56.5) | 43 (59.7) | 18 (50.0) | — |
| >5 cm | 47 (43.5) | 29 (40.3) | 18 (50.0) | — |
| PVTT (%) | 35 (32.4) | 22 (30.6) | 13 (36.1) | 0.7163 |
| Extrahepatic Metastases (%) | 51 (47.2) | 34 (47.2) | 17 (47.2) | 1 |
| Child Pugh (%) | — | — | — | 0.8247 |
| Grade A | 75 (69.4) | 51 (70.8) | 24 (66.7) | — |
| Grade B | 33 (30.6) | 21 (29.2) | 12 (33.3) | — |
| BCLC (%) | — | — | — | 0.8286 |
| Stage B | 36 (33.3) | 23 (31.9) | 13 (36.1) | — |
| Stage C | 72 (66.7) | 49 (68.1) | 23 (63.9) | — |
| AFP (%) | — | — | — | 0.4081 |
| ≤200 ng/mL | 77 (71.3) | 49 (68.1) | 28 (77.8) | — |
| >200 ng/mL | 31 (28.7) | 23 (31.9) | 8 (22.2) | — |

HBV, hepatitis B virus; HCV, hepatitis C virus; HCC, hepatocelluar carcinoma; ECOG PS, eastern cooperative oncology group performance status; TACE, trans-arterial chemoembolization; PVTT, portal vein tumor thrombosis; BCLC, barcelona clinic liver cancer; AFP, alpha-fetoprotein.

interruptions were allowed based on the severity of adverse events (AEs) and tumor progression.

Tumor response was evaluated using mRECIST, and tumor was assessed by contrast computed tomography (CT) or magnetic resonance imaging (MRI). All patients were followed up monthly during the first 6 months of drug treatment and every 3 months after 6 months. The endpoints of this study include ORR, DCR, PFS, OS and safety. ORR was defined as the percentage of complete response (CR) and partial response (PR); DCR was defined as the percentage of CR, PR, and stable disease (SD). PFS is defined as the time interval from initiation of lenvatinib to tumor progression or death, while OS is defined as the time interval from the first dose of lenvatinib to death or last follow-up. Safety was assessed and graded by the Common Terminology Criteria for Adverse Events (CTC-AE, Version 5.0).

## Statistical analyses

All statistical analyses were performed using R software (version 4.0.5). Continuous variables were described using median and range, while categorical variables were expressed as frequency (percentage). In addition, the Mann-Whitney U and Fisher's exact tests were used to compare continuous and categorical variables, respectively. Kaplan-Meier curves for PFS and OS (median, 95% confidence interval (95% CI)) were performed using the log-rank test to detect the differences between the groups. Propensity score matching (PSM) according to virus species was carried out to control for selection bias, confounding factors included age, gender, PVTT, metastasis and Child-Pugh grade. Univariate and multivariate Cox regression were conducted in matched patients to explore independent factors, and subgroup

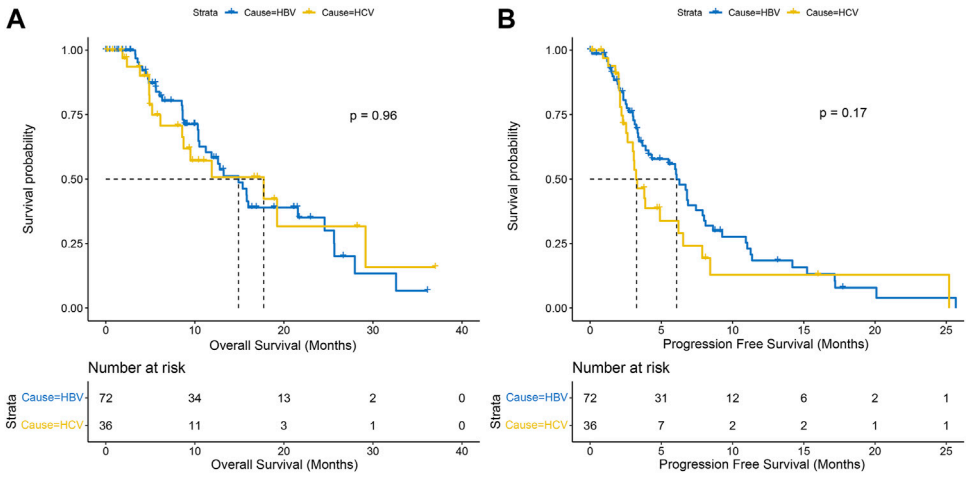


FIGURE 2
Kaplan-Meier curves of overall survival **(A)** and progression-free survival **(B)** between HBV-HCC and HCV-HCC.

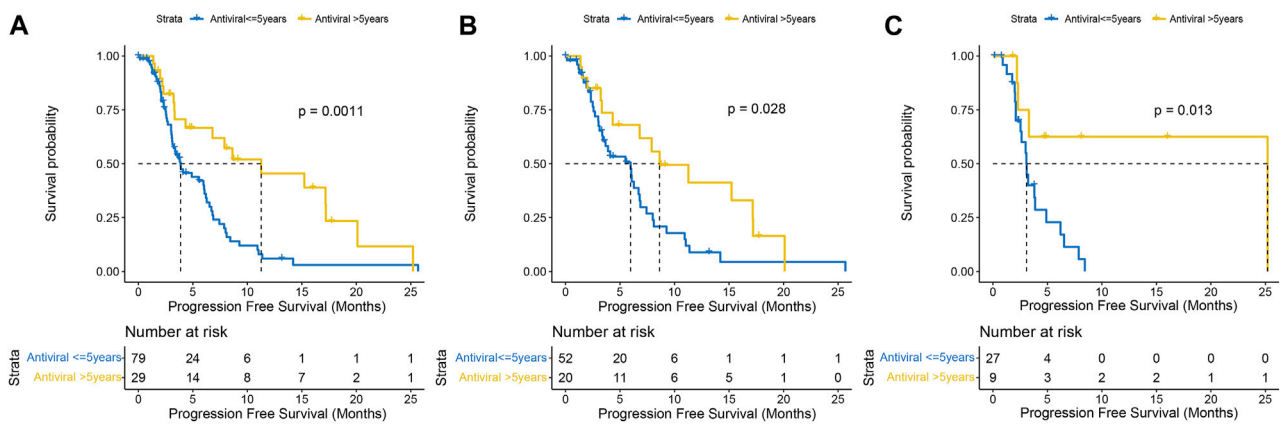


FIGURE 3
Kaplan-Meier curves of progression-free survival with antiviral therapy earlier than 5 years *versus* less than 5 years in the whole population **(A)** HBV-HCC **(B)** and HCV-HCC **(C)**.

analysis was to select patients who would like to benefit more. Statistical significance was set at $p < .05$.

# Results

## Patient characteristics

From October 2017 to October 2021, a total of 203 eligible patients with hepatitis virus-related HCC were treated with mono-lenvatinib, including 163 with HBV-HCC and the remaining 40 with HCV-HCC. After PSM, 72 HBV-HCC and 36 HCV-HCC constituted the study cohort. Figure 1 presents the study cohort selection process. Table 1 summarizes the baseline characteristics of the study population after matching. The differences were eliminated by PSM and balanced and comparable between the two groups. The majority of the PSM populations were males (89.8%), the medium age of the patients was 63.5 years (range: 56.0–69.0 years). Half of the patients had more than 3 tumors, 47 (43.5%) had maximum tumor diameter >5 cm, and the number of patients with PVTT and extrahepatic metastasis was 35 (32.4%) and 51 (47.2%), respectively. Most patients received previous TACE (88.9%), about half received ablation (45.4%), while a few received hepatectomy (14.8%). In addition, 33 (30.6%) patients had Child-Pugh grade B and 72 (66.7%) patients had BCLC stage C.

## Survival analysis

With a median follow-up of 15.6 months, a total of 52 (48.1%) patients died and 76 (70.3%) patients progressed in the matched population, with no significant difference in OS (14.9 months vs. 17.7 months, $p = .96$) and PFS (6.1 months vs. 3.3 months, $p = .17$) between the HBV-HCC and HCV-HCC groups (Figures 2A, B).

**TABLE 2** Cox proportional hazards model of prognostic factors for PFS.

| Characteristics | Univariate analysis | | Multivariate analysis | |
|---|---|---|---|---|
| | P | HR (95%CI) | P | HR (95%CI) |
| Age (>60 years vs. ≤ 60 years) | 0.157 | 1.41 (0.88–2.25) | 0.4532 | 1.21 (0.74–1.99) |
| Sex (male vs. female) | 0.864 | 0.94 (0.45–1.96) | — | — |
| Cause (HBV vs. HCV) | 0.171 | 0.88 (0.43–1.16) | 0.0332 | 0.54 (0.31–0.95) |
| Antiviral (>5 years vs. ≤ 5 years) | 0.001 | 0.41 (0.23–0.71) | 0.0219 | 0.49 (0.26–0.9) |
| Cirrhosis (Yes vs. No) | 0.903 | 1.04 (0.59–1.83) | — | — |
| Surgery (Yes vs. No) | 0.047 | 0.5 (0.25–0.99) | 0.1799 | 0.59 (0.27–1.28) |
| TACE (Yes vs. No) | 0.904 | 1.06 (0.39–2.93) | — | — |
| Ablation (Yes vs. No) | 0.629 | 0.89 (0.57–1.41) | — | — |
| Number (>3 vs. ≤ 3) | 0.462 | 1.19 (0.75–1.87) | — | — |
| Size (>5 cm vs. ≤ 5 cm) | 0.077 | 1.51 (0.96–2.39) | 0.2572 | 1.34 (0.81–2.21) |
| PVTT (Yes vs. No) | 0.168 | 1.4 (0.87–2.26) | 0.8855 | 1.04 (0.63–1.72) |
| Metastases (Yes vs. No) | 0.572 | 1.14 (0.72–1.81) | — | — |
| Child-Pugh (B vs. A) | 0.231 | 1.36 (0.82–2.27) | — | — |
| BCLC (C vs. B) | 0.61 | 1.14 (0.7–1.85) | — | — |
| AFP (>200 ng/mL vs. ≤ 200 ng/mL) | 0.078 | 1.55 (0.95–2.53) | 0.0216 | 1.88 (1.1–3.22) |

PFS, progression-free survival; HR (95%CI), hazard ratio (95% confidence interval); HCV, hepatitis C virus; TACE, trans-arterial chemoembolization; PVTT, portal vein tumor thrombosis; BCLC, barcelona clinic liver cancer; AFP, alpha-fetoprotein.

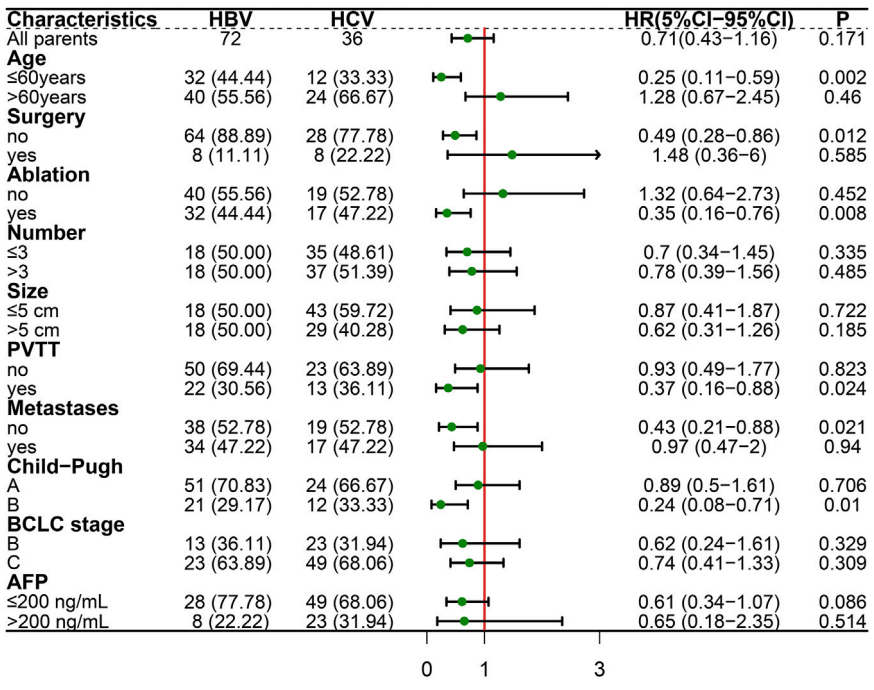


FIGURE 4
Subgroup analysis of progression-free survival in lenvatinib-treated HCC.

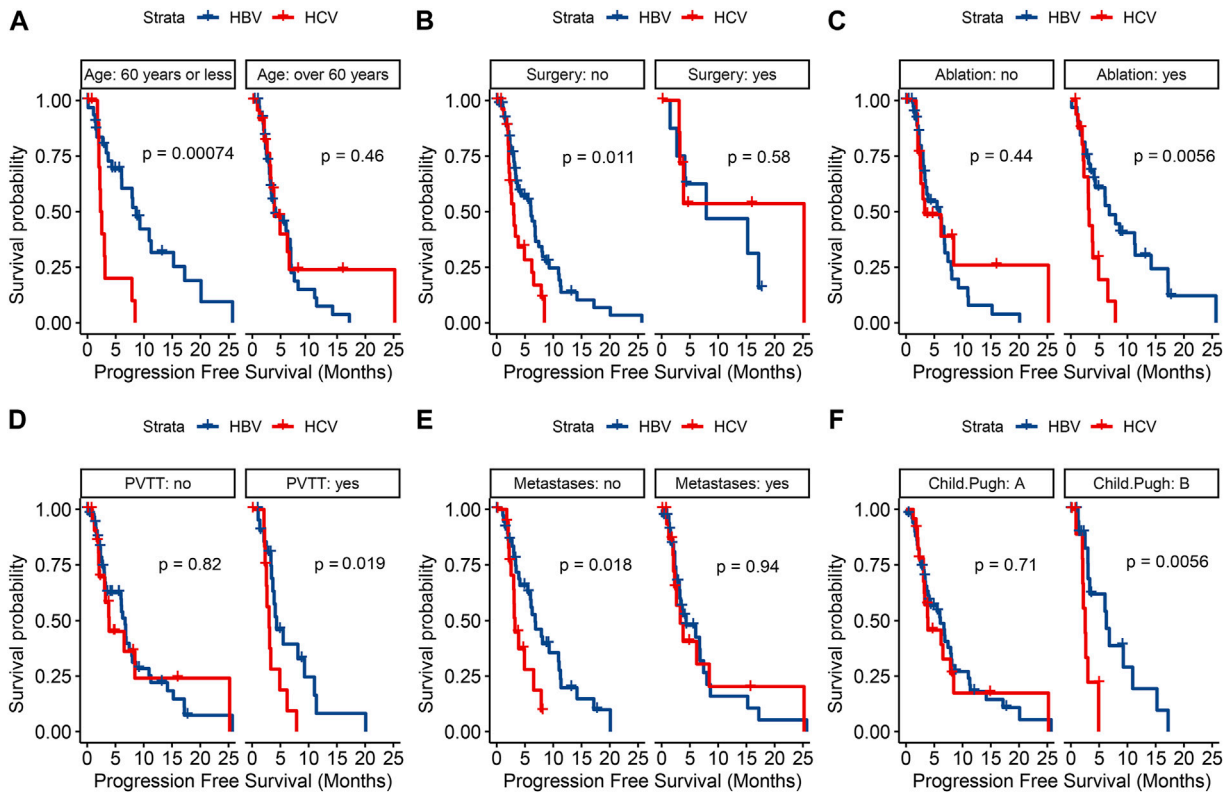


FIGURE 5
Kaplan-Meier curves for progression-free survival in terms of age **(A)** surgical history **(B)** ablation history **(C)** portal vein tumor thrombus **(D)** extrahepatic metastasis **(E)** and Child-Pugh grade **(F)** between the two groups.

Although there was no significant difference, we observed a trend of difference in the Kaplan-Meier curves for PFS.

All 108 patients had undergone antiviral therapy, and the anti-HBV treatments were mainly emptecavir, lamivudine, telbivudine, or teenofovir disoproxil fumarate (TDF), while anti-HCV was interferon plus ribavirin before the DAA era, followed by interferon-free direct antiviral therapy. As of last follow-up, more than half ($n$ = 42, 58.3%) remained HBV DNA positive in the HBV-related HCC group; whereas most patients ($n$ = 29, 80.6%) achieved sustained viral response (SVR) in the HCV-related HCC group. Median PFS was significantly longer in HCCs who had more than 5 years of initial antiviral therapy than in those who had less than 5 years, regardless of virus and drug type (11.27 months vs. 3.87 months, P = .0011) (Figure 3A). In general, patients with HBV infection are treated lifelong, while patients with HCV infection are treated for 3–6 months. Twenty patients in the HBV-related HCC group had antiviral therapy longer than 5 years, while the remaining 52 had less than 5 years, and the former had a significantly better PFS than the latter (8.63 months vs. 5.97 months, $p$ = .028) (Figure 3B). Prior to lenvatinib treatment, antiviral therapy was administered in all HCV-related HCC patients. Nine patients were more than 5 years from their first antiviral treatment and their disease progressed slowly (25.20 months vs. 3.08 months, $p$ = .013) compared with 27 patients less than 5 years (Figure 3C).

According to mRECIST, DCR was significantly higher in HBV-related HCC group compared to the HCV-related HCC group (76.4% vs. 52.8%, $P$ = .0232). Also, ORR was higher in the HBV-related HCC than in the HCV-related HCC (20.8% vs. 5.6%, P = .0759). Within the HBV-related HCC group, 20.8% (n = 15) subjects achieved PR, 55.6% (n = 40) had SD, and 23.6% (n = 17) had progressive disease (PD). While, in the HCV-related HCC group, 5.6% (n = 2) participants achieved PR, 47.2% (n = 17) had SD, and 47.2% (n = 15) had PD.

## Analysis of factors affecting progression

Univariate analysis of PFS showed that age >60 years, HCV infection, antiviral time >5 years, absence of previous surgery, maximum tumor diameter >5 cm, presence of PVTT and alpha-fetoprotein (AFP) > 200 ng/mL were associated with progression in patients treated with lenvatinib (Table 2). Further multivariate analysis, both HBV infection (HR, .54; 95% CI, .31–.95; P = .0332) and antiviral time >5 years (HR, .49; 95% CI, .26–.9; P = .0219) were found to be independent protective factors, and AFP >200 ng/mL (HR, 1.88; 95% CI, 1.1–3.22; P = .0216) was the independent fisk factor for predicting HCC progression. PFS was analyzed in both HBV-HCC and HCV-HCC groups (Figure 4), and the results highlighted HBV-related HCC with age ≤60 years (HR, .25; 95% CI, .11–.59; P = .002), no history of surgery (HR, .49; 95% CI, .28–.86; P = .012), history of ablation (HR, .35; 95% CI, .16–.76; P = .008), presence of PVTT (HR, .37; 95% CI, .16–.88; P = .024), absence of extrahepatic metastases (HR, .43; 95% CI, .21–.88; P = .021), and Child-Pugh grade B (HR, .24; 95% CI, .08–.71; P = .01) had a

**TABLE 3 Treatment related adverse events.**

| Adverse events | All (%) | HBV-HCC (%) | HCV-HCC (%) | P |
|---|---|---|---|---|
| Any grade AEs | 85 (78.7) | 59 (81.9) | 26 (72.2) | 0.3607 |
| Hypertension | 47 (43.5) | 32 (44.4) | 15 (41.7) | 0.9453 |
| Diarrhea | 20 (18.5) | 14 (19.4) | 6 (16.7) | 0.9302 |
| Fatigue | 22 (20.4) | 13 (18.1) | 9 (25.0) | 0.5543 |
| Decreased appetite | 14 (13.0) | 8 (11.1) | 6 (16.7) | 0.6126 |
| Rash | 11 (10.2) | 8 (11.1) | 3 (8.3) | 0.9104 |
| Proteinuria | 8 (7.4) | 5 (6.9) | 3 (8.3) | 1 |
| Hypothyroidism | 7 (6.5) | 5 (6.9) | 2 (5.6) | 1 |
| Elevated transaminase | 5 (4.6) | 2 (2.8) | 3 (8.3) | 0.4182 |
| Nausea/vomiting | 4 (3.7) | 1 (1.4) | 3 (8.3) | 0.2073 |
| Hyperbilirubinemia | 3 (2.8) | 2 (2.8) | 1 (2.8) | 1 |
| Thrombocytopenia | 3 (2.8) | 1 (1.4) | 2 (5.6) | 0.5431 |
| Peripheral edema | 3 (2.8) | 3 (4.2) | 0 (0.0) | 0.5278 |
| Abdominal pain | 3 (2.8) | 3 (4.2) | 0 (0.0) | 0.5346 |
| Hepatic encephalopathy | 3 (2.8) | 2 (2.8) | 1 (2.8) | 1 |
| Grade 3 AEs | 23 (21.3) | 16 (22.2) | 7 (19.4) | 0.9338 |
| Diarrhea | 9 (8.3) | 6 (8.3) | 3 (8.3) | 1 |
| Hypertension | 8 (7.4) | 6 (8.3) | 2 (5.6) | 0.8966 |
| Proteinuria | 3 (2.8) | 2 (2.8) | 1 (2.8) | 1 |
| Hepatic encephalopathy | 3 (2.8) | 2 (2.8) | 1 (2.8) | 1 |
| Thrombocytopenia | 2 (1.9) | 1 (1.4) | 1 (2.8) | 1 |
| Hyperbilirubinemia | 2 (1.9) | 2 (2.8) | 0 (0.0) | 0.8008 |
| Elevated transaminase | 1 (0.9) | 1 (1.4) | 0 (0.0) | 1 |
| Severe AEs | 7 (6.5) | 4 (5.6) | 3 (8.3) | 0.8901 |
| Upper gastrointestinal bleeding | 5 (4.6) | 3 (4.2) | 2 (5.6) | 1 |
| Liver failure | 2 (1.9) | 1 (1.4) | 1 (2.8) | 1 |

AE, adverse event.

significantly longer PFS, when compared to HCV-related HCC. And Kaplan-Meier curves of subgroup analysis are shown in Figure 5.

## Safty

As shown in Table 3, all 108 subjects were analyzed for safety, and the incidence of treatment-related AEs was 81.9% in the HBV-related HCC and 72.2% in the HCV-related HCC. The most common AEs of any grades included hypertension (n = 47, 43.5%), diarrhea (n = 20, 18.5%), fatigue (n = 22, 20.4%), decreased appetite (n = 14, 13.0%), and rash (n = 11, 10.3%), and there were no significant differences between the two groups for any types of AEs. Most of the adverse reactions that occurred were mild to moderate, and few (n = 23, 21.3%) were grade 3. Grade 3 AEs occurred in 16 patients in the HBV-related HCC group, including 6 severe diarrhea, 6 hypertension, 2 proteinuria, 2 hepatic encephalopathy, 2 hyperbilirubinemia, 1 thrombocytopenia, and 1 transaminase elevation; while 7 patients had serious AEs in the HCV-related HCC group, including 3 severe diarrhea, 2 hypertension, 1 proteinuria, 1 hepatic encephalopathy, and 1 hypothyroidism. In total, 7 patients reported severe AEs including 5 upper gastrointestinal bleeding and 2 liver failure, all of which were resolved without sequelae. No significant differences were demonstrated in severe AEs between the two groups. No treatment-related deaths were observed during the study.

# Discussion

In this study, we performed a direct comparison between HBV- and HCV-related HCC treated by mono-lenvatinib, and PSM

balanced some confounding factors to reduce the bias present in retrospective studies. We observed that both ORR and DCR were higher in the HBV-related HCC than in the HCV-related HCC. Although neither PFS nor OS reached statistical significance after matching, post-matching PFS showed a trend of difference. Moreover, multivariate analysis of PFS showed that HCV-infected HCC had significantly shorter PFS. Univariate analysis of the etiology is not significant, but multivariate analysis is significant might because HBV-related HCC often has a large tumor (Barazani et al., 2007; Sinn et al., 2014), and the independent role of HBV on progression is only revealed when the etiology and tumor size are included in multivariate analysis, eliminating the effect of tumor size. Although HBV-related HCC has higher invasiveness than HCV-related HCC (Cantarini et al., 2006), this study suggested lenvatinib has a protective effect on delaying disease progression in HBV-related HCC. This was confirmed by a real-world analysis that HCV-related etiology is less effective for lenvatinib in HCC (Rimini et al., 2021). In addition, we found that the prolongation effect of antiviral therapy on PFS. Although the duration of anti-HBV is longer than that of anti-HCV, the survival difference was observed in both HBV-related HCC group and HCV-related HCC group.

Although chronic HBV and HCV infection are both the main causes of HCC, there are some differences in the mode of transmission, risk factors and carcinogenic mechanisms (Ng and Wu, 2012). HBV, as a DNA virus, can integrate into the hepatocyte genome, mainly through vertical transmission, and serum DNA level and hepatitis B e antigen (HBe Ag) represent active HBV replication (Chen et al., 2006); while HCV is an RNA virus, mainly through blood transmission, and serum RNA level and viral genotype 1b are its risk factors (Ahmad et al., 2011). In addition, HCC caused by HBV and HCV also differ in clinical manifestations and prognosis (Ng and Wu, 2012), and HBV-infected patients are younger at diagnosis of HCC, and often have larger tumors and PVTT, are more likely to be in advanced stages of the disease, while HCV-induced HCC has poor liver function (Barazani et al., 2007; Sinn et al., 2014). The survival outcomes of the two virus-associated HCC differed in several studies, possibly due to differences in patient baseline characteristics, disease stage and treatment modalities (Cantarini et al., 2006; Barazani et al., 2007; Sinn et al., 2014). Contrast to those results, in our present study, the differences in the prognosis were not detected between the HBV-HCC and HCV-HCC. Also, a meta-analysis showed that there were no differences in OS and disease-free survival (DFS) between the HBV and HCV group (Zhou et al., 2011). The underlying reason in our study maybe that due to the use of PSM, there was no difference in age, tumor size, PVTT and liver function between the above two groups. Subgroup analysis of PFS identified a patient population likely to benefit from lenvatinib treatment. Of note, patients with PVTT and Child-Pugh grade B had a significantly worse prognosis in HCV-infected patients, suggesting lenvatinib monotherapy is poorly effective in these patients and may require systemic therapy replacement. Because HCV-infected patients have worse liver function and patients with Child-Pugh grade B are excluded from the REFLECT trial, more studies are needed to investigate its efficacy and safety (Wong et al., 2011; Sinn et al., 2014).

Most HCC do not show clinical symptoms until they progress to an advanced stage, patients have a poor prognosis, and effective systemic therapy is highly warranted (Forner et al., 2018; Villanueva, 2019). Despite great progress in targeted therapy and immunotherapy in recent years, sorafenib and lenvatinib are currently the standard first-line treatments in clinical practice, while the therapeutic response to targeted drugs is related to viral species. Sorafenib has a survival advantage in HCV-infected patients (Bruix et al., 2017; Jackson et al., 2017), which may be due to the fact that sorafenib can inhibit viral replication and reduce the rate of tumor growth and the deterioration degree of liver function (Himmelsbach et al., 2009; Kolamunnage-Dona et al., 2021). Compared with sorafenib, lenvatinib targets are more concentrated and inhibitory. Indirect comparison showed superior short-term efficacy of lenvatinib, second only to atezolizumab combined with bevacizumab in PFS (Park et al., 2019). HBV infection is associated with favorable prognosis of lenvatinib (Kudo et al., 2018; Park et al., 2019), the mechanism of which is unknown, may result in differential drug response due to different molecular mechanisms of HCC etiology, and may also be associated with lenvatinib modulation of the immune microenvironment (Kato et al., 2019). As an indispensable cornerstone drug for HCC, it is crucial to find reliable biomarkers (such as etiology) and predict their therapeutic response (Doycheva and Thuluvath, 2019).

In addition to the etiology, we observed that serum AFP levels had a role in HCC progression. Serum AFP level is the most commonly used biomarker for evaluating the prognosis of HCC. A multicenter study in Japan found that AFP ≥400 ng/mL was an independent risk factor for death (Tsuchiya et al., 2021). The difference was that the cutoff value of this study was 200 ng/mL, and the study outcome was PFS.

This study had some limitations. First, the sample size of HCV group was small, and the observation period was short, with uncontrollable selection bias; second, we excluded HBV and HCV co-infection, which accounted for a small proportion of patients and was not conducive to analysis.

## Conclusion

Compared with HCV-related HCC, the potential benefit of lenvatinib in delaying progression in patients with HBV-related HCC is more pronounced. However, there is a lack of reliable biomarkers for lenvatinib, and we recommend that viral species should be considered in clinical practice, or stratification by etiology in clinical trials.

## Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

## Ethics statement

The studies involving human participants were reviewed and approved by Ethics Committee of Beijing Ditan Hospital, Capital Medical University. The patients/participants provided their written informed consent to participate in this study.

## Author contributions

Study conception and design, JC and WL; Conception, data collection, assembly of data, project administration and manuscript preparation, XL and JW; Data collection, data analysis and manuscript review, XD, YX, MY, HW, and ND. All authors contributed to the article, and approved the submitted version.

## Acknowledgments

We thank all participants for their endeavour and contribution to this study.

## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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## References

Ahmad, J., Eng, F. J., and Branch, A. D. (2011). HCV and HCC: Clinical update and a review of HCC-associated viral mutations in the core gene. *Seminars liver Dis.* 31 (4), 347–355. doi:10.1055/s-0031-1297924

Barazani, Y., Hiatt, J. R., Tong, M. J., and Busuttil, R. W. (2007). Chronic viral hepatitis and hepatocellular carcinoma. *World J. Surg.* 31 (6), 1243–1248. doi:10.1007/s00268-007-9041-3

Bruix, J., Cheng, A. L., Meinhardt, G., Nakajima, K., De Sanctis, Y., and Llovet, J. (2017). Prognostic factors and predictors of sorafenib benefit in patients with hepatocellular carcinoma: Analysis of two phase III studies. *J. hepatology* 67 (5), 999–1008. doi:10.1016/j.jhep.2017.06.026

Cantarini, M. C., Trevisani, F., Morselli-Labate, A. M., Rapaccini, G., Farinati, F., Del Poggio, P., et al. (2006). Effect of the etiology of viral cirrhosis on the survival of patients with hepatocellular carcinoma. *Am. J. gastroenterology* 101 (1), 91–98. doi:10.1111/j.1572-0241.2006.00364.x

Chen, C. J., Yang, H. I., Su, J., Jen, C. L., You, S. L., Lu, S. N., et al. (2006). Risk of hepatocellular carcinoma across a biological gradient of serum Hepatitis B virus DNA level. *Jama* 295 (1), 65–73. doi:10.1001/jama.295.1.65

Cheng, A. L., Kang, Y. K., Chen, Z., Tsao, C. J., Qin, S., Kim, J. S., et al. (2009). Efficacy and safety of sorafenib in patients in the asia-pacific region with advanced hepatocellular carcinoma: A phase III randomised, double-blind, placebo-controlled trial. *Lancet Oncol.* 10 (1), 25–34. doi:10.1016/S1470-2045(08)70285-7

Cooke, G. S., Andrieux-Meyer, I., Applegate, T. L., Atun, R., Burry, J. R., Cheinquer, H., et al. (2019). Accelerating the elimination of viral hepatitis: A lancet gastroenterology and hepatology commission. *lancet Gastroenterology hepatology* 4 (2), 135–184. doi:10.1016/S2468-1253(18)30270-X

Doycheva, I., and Thuluvath, P. J. (2019). Systemic therapy for advanced hepatocellular carcinoma: An update of a rapidly evolving field. *J. Clin. Exp. hepatology* 9 (5), 588–596. doi:10.1016/j.jceh.2019.07.012

Forner, A., Reig, M., and Bruix, J. (2018). Hepatocellular carcinoma. *Lancet (London, Engl.* 391 (10127), 1301–1314. doi:10.1016/S0140-6736(18)30010-2

Himmelsbach, K., Sauter, D., Baumert, T. F., Ludwig, L., Blum, H. E., and Hildt, E. (2009). New aspects of an anti-tumour drug: Sorafenib efficiently inhibits HCV replication. *Gut* 58 (12), 1644–1653. doi:10.1136/gut.2009.182212

Ikeda, K., Kudo, M., Kawazoe, S., Osaki, Y., Ikeda, M., Okusaka, T., et al. (2017). Phase 2 study of lenvatinib in patients with advanced hepatocellular carcinoma. *J. gastroenterology* 52 (4), 512–519. doi:10.1007/s00535-016-1263-4

Jackson, R., Psarelli, E. E., Berhane, S., Khan, H., and Johnson, P. (2017). Impact of viral status on survival in patients receiving sorafenib for advanced hepatocellular cancer: A meta-analysis of randomized phase III trials. *J. Clin. Oncol.* 35 (6), 622–628. doi:10.1200/JCO.2016.69.5197

Kato, Y., Tabata, K., Kimura, T., Yachie-Kinoshita, A., Ozawa, Y., Yamada, K., et al. (2019). Lenvatinib plus anti-PD-1 antibody combination treatment activates CD8+ T cells through reduction of tumor-associated macrophage and activation of the interferon pathway. *PloS one* 14 (2), e0212513. doi:10.1371/journal.pone.0212513

Kolamunnage-Dona, R., Berhane, S., Potts, H., Williams, E. H., Tanner, J., Janowitz, T., et al. (2021). Sorafenib is associated with a reduced rate of tumour growth and liver function deterioration in HCV-induced hepatocellular carcinoma. *J. hepatology* 75 (4), 879–887. doi:10.1016/j.jhep.2021.05.015

Kudo, M., Finn, R. S., Qin, S., Han, K. H., Ikeda, K., Piscaglia, F., et al. (2018). Lenvatinib versus sorafenib in first-line treatment of patients with unresectable hepatocellular carcinoma: A randomised phase 3 non-inferiority trial. *Lancet (London, Engl.* 391 (10126), 1163–1173. doi:10.1016/S0140-6736(18)30207-1

Lanini, S., Easterbrook, P. J., Zumla, A., and Ippolito, G. (2016). Hepatitis C: Global epidemiology and strategies for control. *Clin. Microbiol. Infect.* 22 (10), 833–838. doi:10.1016/j.cmi.2016.07.035

Lemoine, M., Shimakawa, Y., Njie, R., Taal, M., Ndow, G., Chemin, I., et al. (2016). Acceptability and feasibility of a screen-and-treat programme for Hepatitis B virus infection in the Gambia: The prevention of liver fibrosis and cancer in Africa (PROLIFICA) study. *Lancet Glob. health* 4 (8), e559–e567. doi:10.1016/S2214-109X(16)30130-9

Lencioni, R., Montal, R., Torres, F., Park, J. W., Decaens, T., Raoul, J. L., et al. (2017). Objective response by mRECIST as a predictor and potential surrogate end-point of overall survival in advanced HCC. *J. hepatology* 66 (6), 1166–1172. doi:10.1016/j.jhep.2017.01.012

Llovet, J. M., Ricci, S., Mazzaferro, V., Hilgard, P., Gane, E., Blanc, J. F., et al. (2008). Sorafenib in advanced hepatocellular carcinoma. *N. Engl. J. Med.* 359 (4), 378–390. doi:10.1056/NEJMoa0708857

Ng, J., and Wu, J. (2012). Hepatitis B- and hepatitis C-related hepatocellular carcinomas in the United States: Similarities and differences. *Hepat. Mon.* 12, e7635. doi:10.5812/hepatmon.7635

Park, J., Cho, J., Lim, J. H., Lee, M. H., and Kim, J. (2019). Relative efficacy of systemic treatments for patients with advanced hepatocellular carcinoma according to viral status: A systematic review and network meta-analysis. *Target. Oncol.* 14 (4), 395–403. doi:10.1007/s11523-019-00651-7

Peixoto, R. D., Renouf, D. J., Gill, S., Cheung, W. Y., and Lim, H. J. (2014). Relationship of ethnicity and overall survival in patients treated with sorafenib for advanced hepatocellular carcinoma. *J. Gastrointest. Oncol.* 5 (4), 259–264. doi:10.3978/j.issn.2078-6891.2014.036

Petrick, J. L., Florio, A. A., Znaor, A., Ruggieri, D., Laversanne, M., Alvarez, C. S., et al. (2020). International trends in hepatocellular carcinoma incidence, 1978-2012. *Int. J. cancer* 147 (2), 317–330. doi:10.1002/ijc.32723

Rimini, M., Shimose, S., Lonardi, S., Tada, T., Masi, G., Iwamoto, H., et al. (2021). Lenvatinib versus sorafenib as first-line treatment in hepatocellular carcinoma: A multi-institutional matched case-control study. *Hepatology Res. official J. Jpn. Soc. Hepatology* 51 (12), 1229–1241. doi:10.1111/hepr.13718

Sinn, D. H., Gwak, G. Y., Cho, J., Paik, S. W., and Yoo, B. C. (2014). Comparison of clinical manifestations and outcomes between Hepatitis B virus- and hepatitis C virus-related hepatocellular carcinoma: Analysis of a nationwide cohort. *PloS one* 9 (11), e112184. doi:10.1371/journal.pone.0112184

Sung, H., Ferlay, J., Siegel, R. L., Laversanne, M., Soerjomataram, I., Jemal, A., et al. (2021). Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA a cancer J. Clin.* 71 (3), 209–249. doi:10.3322/caac.21660

Tsuchiya, K., Kurosaki, M., Sakamoto, A., Marusawa, H., Kojima, Y., Hasebe, C., et al. (2021). The real-world data in Japanese patients with unresectable hepatocellular carcinoma treated with lenvatinib from a nationwide multicenter study. *Cancers* 13 (11), 2608. doi:10.3390/cancers13112608

Villanueva, A. (2019). Hepatocellular carcinoma. *N. Engl. J. Med.* 380 (15), 1450–1462. doi:10.1056/NEJMra1713263

Wong, P. Y., Xia, V., Imagawa, D. K., Hoefs, J., and Hu, K. Q. (2011). Clinical presentation of hepatocellular carcinoma (HCC) in Asian-Americans versus non-Asian-Americans. *J. Immigr. minority health* 13 (5), 842–848. doi:10.1007/s10903-010-9395-8

Zhou, Y., Si, X., Wu, L., Su, X., Li, B., and Zhang, Z. (2011). Influence of viral hepatitis status on prognosis in patients undergoing hepatic resection for hepatocellular carcinoma: A meta-analysis of observational studies. *World J. Surg. Oncol.* 9, 108. doi:10.1186/1477-7819-9-108

frontiers | Frontiers in Pharmacology

TYPE Review
PUBLISHED 06 February 2023
DOI 10.3389/fphar.2023.1086813



OPEN ACCESS

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SPECIALTY SECTION
This article was submitted to Gastrointestinal and Hepatic Pharmacology, a section of the journal Frontiers in Pharmacology

RECEIVED 01 November 2022
ACCEPTED 18 January 2023
PUBLISHED 06 February 2023

CITATION
Xiao C, Liu S, Ge G, Jiang H, Wang L, Chen Q, Jin C, Mo J, Li J, Wang K, Zhang Q and Zhou J (2023), Roles of hypoxia-inducible factor in hepatocellular carcinoma under local ablation therapies. *Front. Pharmacol.* 14:1086813. doi: 10.3389/fphar.2023.1086813



# Roles of hypoxia-inducible factor in hepatocellular carcinoma under local ablation therapies

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Hepatocellular carcinoma (HCC) is one of the most common digestive malignancies. HCC It ranges as the fifth most common cause of cancer mortality worldwide. While The prognosis of metastatic or advanced HCC is still quite poor. Recently, locoregional treatment, especially local ablation therapies, plays an important role in the treatment of HCC. Radiofrequency ablation (RFA) and high-intensity focused ultrasound (HIFU) ablation are the most common-used methods effective and feasible for treating HCC. However, the molecular mechanisms underlying the actions of ablation in the treatments for HCC and the HCC recurrence after ablation still are poorly understood. Hypoxia-inducible factor (HIF), the key gene switch for adaptive responses to hypoxia, has been found to play an essential role in the rapid aggressive recurrence of HCC after ablation treatment. In this review, we summarized the current evidence of the roles of HIF in the treatment of HCC with ablation. Fifteen relevant studies were included and further analyzed. Among them, three clinical studies suggested that HIF-1α might serve as a crucial role in the RAF treatment of HCC or the local recurrence of HCC after RFA. The remainder included experimental studies demonstrated that HIF-1, 2α might target the different molecules (e.g., BNIP3, CA-IX, and arginase-1) and signaling cascades (e.g., VEGFA/EphA2 pathway), constituting a complex network that promoted HCC invasion and metastasis after ablation. Currently, the inhibitors of HIF have been developed, providing important proof of targeting HIF for the prevention of HCC recurrence after IRFA and HIFU ablation. Further confirmation by prospective clinical and in-depth experimental studies is still warranted to illustrate the effects of HIF in HCC recurrence followed ablation treatment in the future.

KEYWORDS

**hepatocellular carcinoma, hypoxia-inducible factor, ablation, therapy, mechanism**

## Introduction

Hepatocellular carcinoma (HCC) is the third most common cancer and the fifth most common cause of cancer mortality worldwide (Calderaro et al., 2022; Sperandio et al., 2022). The high mortality of HCC is attributed to the lack of early detection and few effective therapies, especially for intermediate- or advanced HCC patients (Parikh and Pillai, 2021). With the development of medical technology, various new treatment techniques have brought new opportunities for HCC treatment. At present, hepatectomy, liver transplantation, and ablation

are the standard treatment for HCC (Zhuang et al., 2021). However, the advanced neoplastic stage and the shortage of donors limit the application of hepatectomy and liver transplantation. Recently, locoregional treatment, especially local ablation therapies, plays an important role in the treatment of HCC.

Local thermal ablation techniques mainly include radiofrequency ablation (RFA), high-intensity focused ultrasound (HIFU) ablation, laser ablation, and microwave ablation (Jing et al., 2020). Among these, RFA and HIFU have been most frequently used worldwide in the treatment of HCC. RFA heats targeted tissue by ionic friction from current, reducing the local increase in temperature above 60 °C and then causing coagulative necrosis (Yousaf et al., 2020). HIFU employs the ultrasonic wave to heat tumor entities, resulting in coagulative necrosis of tumor tissue (Sofuni et al., 2022). Local ablation therapy is more secure and has fewer complications and shorter hospital stays than hepatectomy (Shin et al., 2021). In addition, RFA can be combined with other therapies to treat HCC, thereby providing a better therapeutic effect and overcoming its limitations. For example, a randomized, controlled pilot study reported that RFA combined with TACE showed better effectiveness than RFA alone for HCC (Morimoto et al., 2010). Nevertheless, due to residual viable tumors after local ablation, the rate of recurrence and metastasis is higher than that for surgical resection in HCC. It is reported that the 5-year overall recurrence rates were 63.5% with RFA, while surgical resection was 41.7% in patients with HCC(Huang et al., 2010). As a result, further studies of the molecular mechanism of HCC relapses after local ablation are thus needed so that novel medication targets can be developed.

Local thermal ablation damage has been found to be divided into three regions (central high-temperature zone, sublethal temperature transition zone, and surrounding normal tissue) (Sauvaget et al., 2022). In the transition zone, the damage of tumors can be reversed and eventually survive, thus resulting in the rapid development of tumors. There are increasing studies suggesting that epithelial-mesenchymal transitions (EMT), autophagy, and the hypoxic microenvironment play crucial roles in subsequent progression and metastasis after local ablation. Li et al. (2022) found that insufficient RFA (IRFA) promoted proliferation, invasion, migration, and EMT in HCC cells. Zhao et al. (2018) reported that autophagy has been shown to be activated in mice exposed to IRFA. Importantly, hydroxychloroquine (HCQ), a well-established inhibitor of autophagy, significantly suppressed HCC proliferation and recurrence induced by IRFA (Zhao et al., 2018). Recently, Frenzel et al. (2018) suggested that hypoxia-inducible factor (HIF), the key gene switch for adaptive responses to hypoxia, played an important role in the rapid aggressive recurrence of HCC after RFA. Currently, there is still a lack of narrative reviews on the role of HIF in the local ablation of HCC. In this review, we present the first attempt to comprehensively summarize the recent advances of HIF in local ablation of HCC. The aim of this article is to facilitate the clinical application of HIF in inhibiting the rapid aggressive recurrence of HCC after RFA.

## Overview OF HIF

HIF, a heterodimeric transcription factor, plays a pivotal role in the ability to adapt to changes in oxygen levels (Kling et al., 2021). HIF was first described by Semenza and others in 1995 (Wang et al., 1995; Wang and Semenza, 1995). In 1991, he found a hypoxic inducible nuclear factor, bounding to the promoter of the EPO gene, and then increasing its expression (Semenza et al., 1991). This nuclear factor has been named HIF by Semenza and others in 1995 (Wang et al., 1995; Wang and Semenza, 1995). The huge importance of this discovery was reflected by the 2019 award of the Nobel Prize in Physiology or Medicine (Fitzpatrick, 2019). HIF is composed of α and β subunits. The α subunit is modulated in an oxygen-dependent manner but the β subunit is constitutively expressed (Macedo-Silva et al., 2020). There are three variants of the α subunit, i.e., HIF-1α, HIF-2α, and HIF-3α, and three paralogues of the β subunit, i.e., HIF-1β, HIF-2β, and HIF-3β (Tsai et al., 2020). HIF-1α is the most ubiquitously expressed of the three isoforms and has 48% amino acid sequence identity to HIF-2α (Fitzpatrick, 2019). It has been reported that HIF-1α and HIF-2α played a key role in the acute and chronic response to hypoxia, respectively (Galan-Cobo et al., 2015; Guo et al., 2021). Due to the existence of multiple HIF-3α variants, it is less well characterized. HIF-α is regulated by oxygen-dependent pathways. The HIF-α subunits are synthesized at a very high rate but undergo rapid degradation *via* oxygen-dependent prolyl hydroxylase (PHD) enzymes in the presence of oxygen (Strowitzki et al., 2019). The PHD enzymes could hydroxylate Pro402 and Pro564, two conserved proline residues (Snell et al., 2014). The post-translational hydroxylation enables the von Hipple-Lindau (VHL) to bind the HIF-α subunit for degradation (Liu et al., 2018). The human genome encodes three types of PHD enzymes, i.e., PHD1, 2, and 3 (Fujita et al., 2012). It has been reported that PHD2 regulated HIF-1α, while PHD1 regulated HIF-2α(Ren et al., 2011; Vara-Perez et al., 2021). As oxygen availability decrease, the PHDs activity is diminished, leading to the translocation of HIF-α into the nucleus (Nguyen et al., 2021). HIF-α binds to a hypoxia response element in the nucleus and results in the activation of target genes, which facilitates adaptation and survival of cells and contributes to angiogenesis, proliferation, and metastasis of the tumor (Kaelin and Ratcliffe, 2008).

Mounting evidence demonstrates that HIF may correlate with numerous human diseases (e.g., breast cancer, cervix cancer, and HCC) (Feng et al., 2020; Lal et al., 2021). As known, hypoxia is a feature of most solid tumors, where the oxygen level is usually below 1% (D'Ignazio et al., 2017). A series of reactions is caused by hypoxia, which affects tumor survival and progression and confers resistance to chemoradiotherapy by influencing angiogenesis and metabolism (Muz et al., 2015). HIF has been shown to be the main regulator of these responses (Rocha, 2007). Previous studies have shown that the expression levels of HIF-1α and/or HIF-2α were increased in various tumors such as breast cancer, prostate cancer, and pancreatic cancer, and were correlated with poor survival (Shaida et al., 2008; Zhang et al., 2017; De Francesco et al., 2018; Zhang H. S et al., 2019). Also (Hu et al., 2021), demonstrated that hypoxia significantly induced high expression of HIF-1α and HIF-2α, which promoted the proliferation, migration, and invasion of HCC cells. It was reported that the enhancement of HIF activity promoted tumor metastasis through the regulation of hundreds of genes related to immune escape, cancer stem cell maintenance, angiogenesis, and EMT (Rankin and Giaccia, 2016; Schito and Semenza, 2016). In addition to regulation by PHD and VHL mentioned above, the expression of HIF is also regulated by inflammation and epigenetic regulator in HCC. In the presence of persistent hypoxia, IL-1β is released by the necrotic

debris of HCC cells, which promotes HIF-1α synthesis *via* cyclooxygenase 2 (COX-2) (Zhang et al., 2018). The histone deacetylase 5 (HDAC5), an epigenetic regulator, is essential for activating gene transcription and is also involved in the development and progression of tumors (Zhou et al., 2021a). The expression of HDAC5 has been suggested to be elevated in HCC tissues and cells compared with non-cancerous HCC tissues and control cells (Feng et al., 2014). The elevated expression of HDAC5 can induce the transcription of HIF-1α and lead to high expression of HIF-1α by silencing homeodomain-interacting protein kinase-2 (HIPK2) (Ye et al., 2017).

Studies have shown a close association between HIF and recurrence after ablation (Ikemoto et al., 2017). Previous studies indicated that ablation caused hypoxia adjacent to the ablated area, which is related to tumor proliferation, angiogenesis, and aggressiveness (Wan et al., 2016a; Gong et al., 2019). Moreover, the hypoxic status induced by ablation elicits HIF accumulation (Zhang J et al., 2021). (Wan et al., 2016b) discovered that the HSP70/HIF-1α signaling pathway was significantly enhanced in hyperthermia-induced lung cancer cells. Meanwhile, hyperthermia-induced lung cancer cells possess higher proliferation and angiogenesis potential (Wan et al., 2016a). The analogous result was observed in HCC. Kong et al. (2012) found that the expression level of HIF-1α was upregulated in heat-treated HCC cells. Also, a stronger pro-angiogenic effect was observed in heat-treated HCC cells (Kong et al., 2012). Importantly, this effect could be inhibited by HIF-1a inhibitor YC-1 (Kong et al., 2012). These results suggested that HIF may play a crucial role in the rapid progression of residual cancer after ablation.

## Literature search

To maximally identify the eligible articles related to the role of HIF in HCC after ablation, we carried out a systematic search of MEDLINE, Google Scholar, EMBASE, and Cochrane Library databases. The search keyword in MEDLINE was: ((((((((((((((((((((“Carcinoma, Hepatocellular" [Mesh]) OR (Carcinomas, Hepatocellular)) OR (Hepatocellular Carcinomas)) OR (Liver Cell Carcinoma, Adult)) OR (Liver Cancer, Adult)) OR (Adult Liver Cancer)) OR (Adult Liver Cancers)) OR (Cancer, Adult Liver)) OR (Cancers, Adult Liver)) OR (Liver Cancers, Adult)) OR (Liver Cell Carcinoma)) OR (Carcinoma, Liver Cell)) OR (Carcinomas, Liver Cell)) OR (Cell Carcinoma, Liver)) OR (Cell Carcinomas, Liver)) OR (Liver Cell Carcinomas)) OR (Hepatocellular Carcinoma)) OR (Hepatoma)) OR (Hepatomas)) AND (((Hypoxia Inducible Factor) OR (Hypoxia-Inducible Factor)) OR (HIF))) AND (Ablation). The inclusion criteria are as follows: 1) clinical study reporting on the roles of HIF in HCC after ablation; 2) experimental research reporting on the effects of HIF in HCC after ablation and its possible molecular mechanisms. Finally, fifteen clinical or experimental studies were included for further analysis. The researchers then used a specific data collection table to extract relevant data from each study, including the general information of the article (e.g., the first author’s name, the year of publication), research subject (e.g., cell/animal model or patient), ablation surgical type, associated genes/pathways and agents, measure method of HIF, and the major findings of the study. Table 1 summarizes the relevant studies reporting the roles of HIF on HCC after ablation.

# Clinical implications of HIF-1α in HCC after ablation

Among the 15 included studies, three clinical studies reported the clinical roles of HIF-1α on HCC after ablation. Yamada et al. (2014) conducted a clinical study of Eighty-eight (88) patients with HCC. These patients were categorized into two groups (i.e., RFA group and non-RFA group). There was no significant difference in TMN staging, number of tumors, maximum tumor diameter, and tumor markers between the two groups. The overall 5-year survival rate of the RFA groups was lower than those of non-RFA groups (39% vs. 68%; $p$ = 003). As identified through qRT-PCR, the RFA group showed increased expression not only of HIF-1α, but also of epithelial cell adhesion molecule (EpCAM), a marker for tumor cell growth and metastasis, compared to the non-RFA group. Of note, the expression level of angiogenic factors VEGF was not different between the two groups. Yamada et al.‘s study revealed that the local recurrence of HCC after RFA was significantly associated with the increased expressions of HIF-1 and EpCAM, but not the expressions of VEGF (Yamada et al., 2014). As is well known, sorafenib, an oral multi-kinase inhibitor, can block the proliferation and induce cell apoptosis, which has been widely to treat advanced HCC. Gong et al. (2017) assigned 50 patients with HCC to control group and 40 patients with HCC to observation group. Two groups of patients were treated with conventional RFA alone and with a combination of RFA and oral sorafenib, respectively (Gong et al., 2017). The patients in the observation group showed more prolonged tumor-free survival (12.3 vs. 8.4 months), lower relapse (15.0% vs. 34.0%), and longer survival rates (87.5% vs. 70.0%) than those in the control group (Gong et al., 2017). Furthermore, the overall treatment efficacy in the observation group was superior to that in the control group (82.5% vs. 62.0%); however, the incidence of complications showed no differences between the two groups (17.5% vs. 20.0%) (Gong et al., 2017). The author further found that in comparison to the average levels before treatment, the average levels of serum VEGF and HIF-1α were decreased after the treatment (Gong et al., 2017). This study suggested that sorafenib in combination with RFA was a superior treatment for HCC and HIF-1α and VEGF played key roles during the treatment process. Similarly, another study performed by Yuan et al. (2017) showed that the combination of transcatheter arterial chemoembolization (TACE) and RAF significantly decreased liver cancer angiogenesis and reduced HCC cell proliferation speed than TACE alone. Moreover, the TACE combined with RAF reduced the expression levels of HIF-1α and early growth response protein 2 (EGR-2) to a greater extent than TACE alone (Yuan et al., 2017). In summary, the above three clinical studies suggested that HIF-1α might serve a crucial role in the RAF treatment of HCC or the local recurrence of HCC after RFA.

# The roles of HIF and the related signaling pathway in HCC after ablation

## HIF-1α contributes to the progression of HCC by promoting the expression of VEGFA and arginase-1

Arginase, a pivotal metabolic enzyme, is a part of the urea cycle and catalyzes the hydrolysis of L-arginine, resulting in ammonia

**TABLE 1 The characteristics and the main findings of the included studies.**

| Study/ Reference | Research subject | Ablation surgical type | Measure method | Associated genes/ pathways and agents | Main findings |
|---|---|---|---|---|---|
| Kong et al. (2012) | HCC cells | RFA | Western blot | Up-regulated HIF-1α/VEGFA pathway | RFA promoted the growth of residual HCC by inducing angiogenesis *via* HIF-1α/VEGFA pathway |
| Halpern et al. (2021) | Mice | RFA | Western blot and PCR | Up-regulated HIF-1α/arginase-1 or HIF-1α/VEGF pathway | RFA led to an increase in engraftment and progression of hepatic metastases by enhancing HIF-1α expression |
| Chen et al. (2021) | Nude mice | Microwave ablation | Western blot and PCR | Enhanced the Warburg effect | HIF-1α promoted HCC progression after thermal ablation by enhancing the Warburg effect |
| Wu et al. (2014) | Nude mice | HIFU ablation | Immunohistochemistry, Western blot and PCR | Enhanced the HIF-1, 2α/VEGFA/ EphA2 pathway | HIFU ablation enhanced pro-angiogenic effect by HIF-1, 2α/ VEGFA/EphA2 pathway in the residual hepatocellular carcinoma |
| Wu et al. (2017) | Nude mice | HIFU ablation | Immunohistochemistry and Western blot | Enhanced the HIF-2α/VEGFA/ EphA2 pathway | HIFU ablation induced residual tumor angiogenesis by up-regulating HIF-2α/VEGFA/ EphA2 pathway in HCC. |
| Xu et al. (2013) | Nude mice | RFA | PCR and immunohistochemistry | Up-regulation of HIF-1α/VEGFA pathway | Insufficient RFA promoted recurrence of HCC by increasing HIF-1α and VEGFA expression |
| Nijkamp et al. (2009) | Male BALB/c mice and male Wag/Rij rats | RFA | Western blot and immunohistochemistry | Up-regulation of HIF-1, 2α and downstream markers CA IX and VEGF | RFA induced the outgrowth of tumor cells through the up-regulation of HIF-1, 2α pathways in animal model of colon cancer hepatic metastasis |
| Tong et al. (2017) | HCC cells | RFA | Western blot | HIF-1α induced EMT | HIF-1α increased the migration, invasion, and sorafenib chemoresistance by inducing EMT after RFA. |
| Xu et al. (2019) | HCC cells | RFA | Western blot | Up-regulation of HIF-1α/ BNIP3 pathway | IRFA promoted residual HCC cell progression by enhancing autophagy *via* up-regulation of HIF-1α/BNIP3 pathway |
| Wu et al. (2014) | BALB/c nu/nu mice | HIFU ablation | Immunohistochemistry, Western blot and PCR | Up-regulation of HIF-2α/VEGF pathway | HIFU treatment promoted angiogenesis by up-regulating HIF-2α/VEGFA pathway in mice with hepatocellular carcinoma |
| Wu et al. (2021) | Nude mice | HIFU ablation | Western blot, PCR and immunohistochemistry | Sorafenib inhibited the HIF-2α/ VEGF-A/EphA2 pathway | Sorafenib inhibited HIFU ablation-induced progression of the residual tumor by suppressing HIF-2α/VEGF-A/ EphA2 pathway in HCC. |
| Yamada et al. (2014) | Patients (n = 88) | RFA | PCR | Up-regulation of HIF-1α and EpCAM | The RFA group showed aggressive tumor phenotype and poor prognosis by enhancing HIF-1α and EpCAM expression in the residual HCC tumors |
| Yuan et al. (2017) | Patients (n = 144) | RFA | ELISA | TACE inhibited HIF-1α and EGR2 | TACE combined with RFA reduced tumor cell proliferation speed by inhibiting the expression of HIF-1α |
| Dong et al. (2022) | HCC cells and nude mice | RFA | Western blot and immunohistochemistry | ATO inhibited p-Akt/HIF-1α pathway | ATO inhibited angiogenesis in HCC by blocking Ang-1 and Ang-2 through the inhibition of p-Akt/HIF-1α pathway |

(Continued on following page)

**TABLE 1** (*Continued*) **The characteristics and the main findings of the included studies.**

| Study/ Reference | Research subject | Ablation surgical type | Measure method | Associated genes/ pathways and agents | Main findings |
|---|---|---|---|---|---|
| Gong et al. (2017) | Patients (n = 90) | RFA | ELISA | Sorafenib reduced VEGF, CTGF, HIF-1α and OPN expression | Sorafenib combined with RFA showed a superior overall treatment efficacy than only RFA by inhibiting the expression of VEGF, CTGF, HIF-1α and OPN in HCC. |

Note: HCC = Hepatocellular carcinoma; RFA = Radiofrequency ablation; HIF-1α = Hypoxia inducible factor-1 alpha; VEGF = Vascular endothelial growth factor; VEGFA = Vascular endothelial growth factor A; HIFU = High-intensity focused ultrasound; HIF-2α = Hypoxia inducible factor-2 alpha; EphA2 = Epithelial cell kinase; CA IX = Carbonic anhydrase IX; EMT = Epithelial-mesenchymal transition; BNIP3 = Bcl-2/adenovirus E1B 19 kDa interacting protein 3; EpCAM = Epithelial cell adhesion molecule; TACE = Transcatheter arterial chemoembolization; EGR2 = Early growth response protein 2; ATO = Arsenic trioxide; CTGF = Connective tissue growth factor; OPN = Osteopontin.

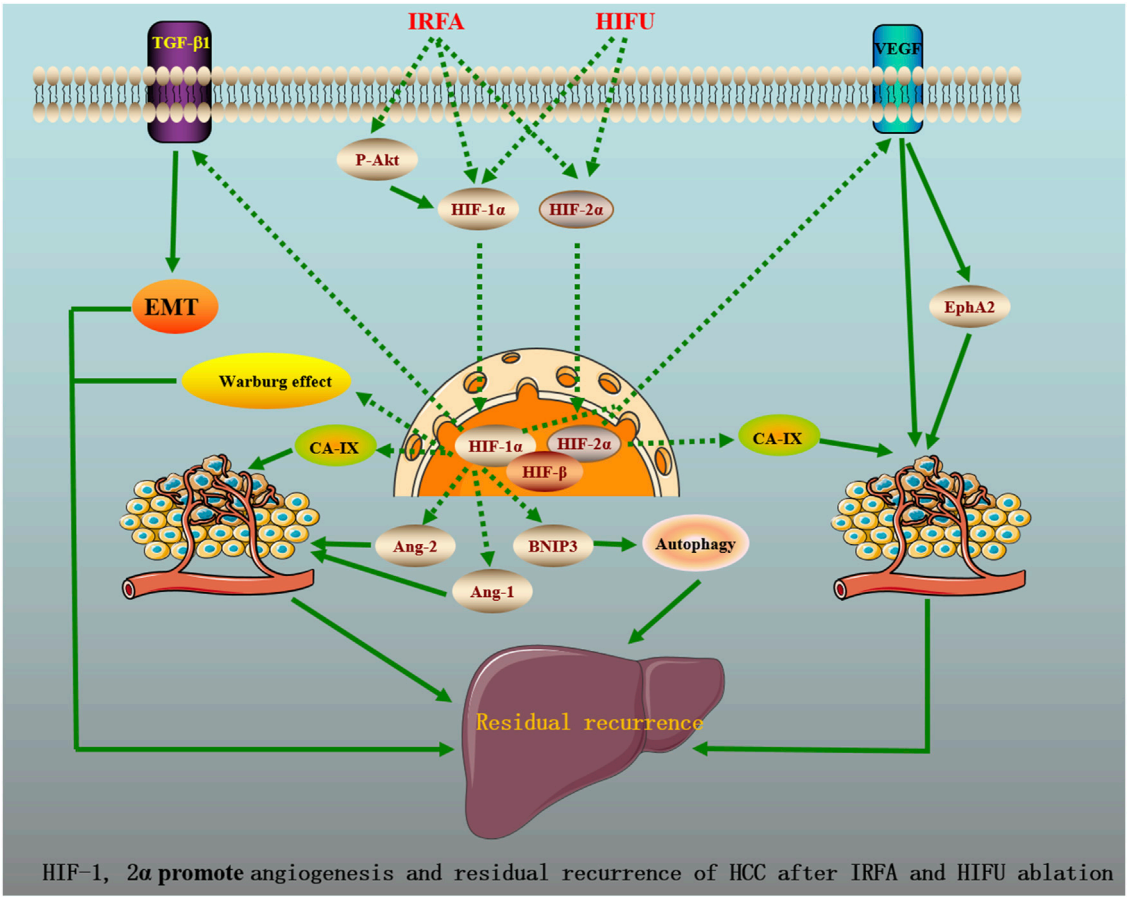


FIGURE 1
HIF-1, 2a promote angiogenesis and residual recurrence of HCC after IRFA and HIFU ablation.

detoxification in mammals (Cziraki et al., 2020). It has two isoforms: arginase-1 and arginase-2, localized in the cytosol and mitochondria, respectively (Labib et al., 2020). Arginase-1 is primarily detected in hepatocytes and can be served as an important marker of hepatocellular differentiation (Moudi et al., 2020). Recently, arginase-1 has been reported to be induced in activated macrophages and participates in the initiation and progression of various diseases, including HCC (You et al., 2018). In addition, HIF-1α is reported to be involved in the expression of the arginase-1 (Alexander et al., 2020). The HIF well-studied target is the vascular endothelial growth factor A (VEGFA) (Palazon et al., 2017). It is the main stimulator of tumor angiogenesis and is reported to be overexpressed in multiple solid cancers, including HCC (Zou et al., 2020). Lin et al. (2018) demonstrated that long non-coding RNA UBE2CP3, a cancer-promoting gene of HCC, enhanced the progression of liver cancer by promoting angiogenesis through the activation of ERK1/2/HIF-1α/VEGFA signaling pathway. A recent study conducted by Halpern et al. (2021) showed that a metastatic tumor model was established using a splenic injection of colon adenocarcinoma cells, and all mice undergoing RFA developed tumors at the ablation site on necropsy performed at 7 days. In contrast, the site of probe insertion had no tumors in the mice

undergoing a sham procedure (Halpern et al., 2021). Furthermore, the expression of HIF-1α and VEGFA was significantly increased at the ablation site relative to unaffected adjacent liver tissues from the same mouse (Halpern et al., 2021). The expression of mRNA of HIF-1α, VEGFA, and arginase-1 were also significantly elevated in RFA/tumor-associated macrophages compared with that in unaffected liver (Halpern et al., 2021). Further study found that the tumor volume and the median number of total metastases were significantly decreased in mice treated with YC-1, a well-established inhibitor of HIF-1α, compared to vehicle control (Halpern et al., 2021). In agreement with the above study, Xu et al. (2013) found that the expression of HIF-1α and VEGFA were significantly increased in hepatic tumor model mice after RFA and this response could be inhibited by the combination of sorafenib and RFA. Therefore, HIF-1α might promote the development of hepatic metastases after RFA by increasing the expression of VEGFA and arginase-1.

HIF-1 and 2α promote angiogenesis and residual recurrence of HCC after HIFU ablation *via* VEGFA/EphA2 pathway.

High-intensity focused ultrasound (HIFU) is another local ablative therapy and is also difficult to achieve complete ablation of HCC, which contributes to the recurrence of HCC. Erythropoietin-producing hepatocellular A2 (EphA2), a member of the Eph family of receptor tyrosine kinases, is a crucial regulator of tumorigenesis and is highly expressed in multiple cancers, including HCC (Wilson et al., 2021). EphA2 has been shown to be activated through the phosphorylation on serine 897 mediated by AKT, RSK, and PKA kinases and alters downstream signaling, and then facilitating tumor progression (Zhou et al., 2015; Barquilla et al., 2016). Niu et al. (2021) reported that EphA2 was highly expressed in HCC. Additionally, EphA2 silencing significantly reduced cell proliferation and accelerated apoptosis (Niu et al., 2021). A study conducted by Cheng et al. (2002) demonstrated that EphA2 antisense oligonucleotides significantly suppressed endothelial expression of the EphA2 receptor and inhibited VEGF-induced cell migration. A recent study showed that the knockdown of HIF-1α by siRNA downregulated the expression of EphA2 and led to apoptosis and the disruption of vasculogenic mimicry (VM) associated phenotypes (Saha et al., 2022). Nevertheless, it is unknown whether EphA2 functions in HCC after HIFU ablation. Wu et al. (2014) investigated the roles of EphA2, VEGFA, and HIF-1α in a xenograft model of HCC in nude mice. The authors found that the protein and mRNA levels of HIF-1α and HIF-2α were significantly increased in the residual tumor tissues of the HIFU group compared to that in the control group (Wu et al., 2014). Furthermore, similar results were obtained for VEGF-A and EphA2 expression (Wu et al., 2014). The expression of CD31 is considered an indicator of calculated the microvascular density (MVD) and is significantly increased compared with the control grou p (Wu et al., 2014). Importantly, the alteration of CD31, VEGF-A, and EphA2 expression were basically consistent with the trends in HIF-1α and HIF-2α expression (Wu et al., 2014). Therefore, the authors suggested HIFU ablation might result in the hypoxia condition of residual tumor and induce tumor angiogenesis *via* HIF-1, 2α/VEGFA/EphA2 (Wu et al., 2014). Two other studies conducted by Wu et al. (2017); Wu et al. (2021) also demonstrated that overexpression of EphA2, VEGFA, and HIF-2α were closely associated with angiogenesis in residual HCC after HIFU ablation. Moreover, the expression of EphA2, VEGFA, and HIF-2α could be significantly inhibited by sorafenib (Wu et al., 2021). Thus, inhibiting HIF-1, 2α alone or in combination with angiogenesis inhibitors might prevent residual recurrence of HCC after HIFU ablation.

## HIF-1α and HIF-2α promote liver metastases of colon carcinoma following RFA *via* the activation of downstream markers CA-IX and VEGF

The induction of angiogenesis is an important biological process in responseto hypoxia (Ben et al., 2022). Also, active pH regulation is an essential biological process in response to hypoxia, which allows tumor cells to maintain viability and proliferation in the acidic tumor microenvironment (Porter and Porter, 2018). Carbonic anhydrase IX (CA IX) has been proven to be a key component of this pH regulatory machinery (Benej et al., 2020). CA IX, a transmembrane protein, catalyzes the reversible dehydration of bicarbonate. In recent studies, CA IX is served as a potent biomarker of poor patient prognosis for many types of solid tumors, including HCC (Parks and Pouyssegur, 2017; Chen et al., 2018). Cho et al. (Cho et al., 2019) demonstrated that CA IX has overexpressed in HCC and CA IX inhibitor (acetazolamide) significantly suppressed the growth of HCC xenograft tumors in nude mice. In addition, HCC patients with high CA IX expression displayed a worse prognosis in the TCGA database (Cho et al., 2019). CA IX has been reported to be a downstream marker of HIF (Nijkamp et al., 2009). Nijkamp et al. (2009) established preclinical models with colorectal micrometastases to investigate the effect of RFA on the outgrowth of tumor cells at the lesion periphery. The authors found that tumor load in the transition zone (TZ) significantly increased after RFA compared with tumor load in the livers of sham-operated mice (48.5 ± 3.9% vs. 17.9 ± 2.0%, $p = 0.00021$) (Nijkamp et al., 2009). Furthermore, tumor load in the TZ following RFA had increased approximately 4-fold compared with tumor load in the reference zone (RZ) (Nijkamp et al., 2009). HIF-1α, HIF-2α, CA-IX, and VEGF were significantly up-regulated in the TZ (Nijkamp et al., 2009). As is noted, 17DMAG, an inhibitor of HIF, suppressed the expression of HIF-1α and HIF-2α, and significantly reduced tumor growth in the TZ (Nijkamp et al., 2009). However, 17DMAG had no obvious effect on tumor growth in the RZ of RFA-treated mice (Nijkamp et al., 2009). The above studies suggested that HIF-1, 2α might promote liver metastases of colon carcinoma following RFA by enhancing the expression of CA-IX and VEGF, the downstream markers of HIF.

## HIF-1α/BNIP3 pathway promotes residual HCC cell progression by enhancing autophagy after IRFA

Autophagy is a highly conserved, intracellular self-protective process, critically required for the degradation of damaged organelles and cytoplasmic material (Verma et al., 2021). There are accumulating data showing that autophagy plays a key role in a variety of tumors, including HCC. In pancreatic cancer, autophagy promoted tumor growth in syngeneic host mice, but the inhibition of autophagy reduced tumor growth by restoring surface levels of major histocompatibility complex class I (Yamamoto et al., 2020). In HCC, the expression of LC3-II, a key autophagic marker, was significantly increased and associated with poor prognosis of HCC. Moreover, inhibition of autophagy reduced the ability of tumor cells to survive (Han et al., 2021; Zhang K et al., 2021). Wang et al. (2018) found that the activity of autophagy was markedly enhanced in the residual HCC cells after RFA and the autophagy inhibitor 3-methyladenine (3-MA) significantly inhibited the cell viability and

**TABLE 2 Main findings of the recent clinical trials in the field of HCC ablation.**

| Study/Reference | Main findings |
|---|---|
| Qiao et al. (2022) | Addition of anti-PD-1 adjuvant therapy after TACE combined with ablation significantly prolong the relapse-free survival with controllable safety for HCC patients with high recurrence risk |
| Wang et al. (2022) | Repeat hepatectomy should be the first choice for solitary small recurrent HCC patients with late recurrence, while MWA should be selected for those with early recurrence |
| Radosevic et al. (2022) | MWA created larger ablation zones than RFA ($p = 0.036$) although without differences in short-to-long diameter ratio of ablation zone. Both MWA and RFA are effectiveness and safety in liver tumors between 1.5 and 4 cm |
| Suh et al. (2021) | No-touch RFA using twin internally cooled wet electrodes demonstrated significantly lower cumulative local tumor progression rates than conventional RFA for small HCCs |
| Iwata et al. (2021) | A phase 2 study demonstrated that image-guided proton therapy (IGPT) is a safe and effective treatment for solitary operable or ablation-treatable HCC |
| Zaitoun et al. (2021) | Combined therapy with conventional TACE + MWA is safe, well-tolerated, and more effective than TACE or MWA alone for treatment of HCC with 3–5 cm |
| Bockorny et al. (2022) | Priming of sorafenib did not enhance the effect of RFA in intermediate sized HCC. |

invasion induced by IRFA. Recently, mounting studies have shown that Bcl-2 19-kDa interacting protein 3 (BNIP3) is served as a mitochondrial protein and plays a critical role in autophagy. For example, Xu et al. (2021) reported that latent membrane protein1 (LMP1) promoted radioresistance by inducing autophagy through BNIP3 in nasopharyngeal carcinoma. Additionally, BNIP3 can be induced by hypoxia and is confirmed to be the target molecule of HIF-1α (Chen W et al., 2016). Zhang Y. et al. (2019) demonstrated that HIF-1α played a protective role against myocardial ischemia-reperfusion injury by inducing BNIP3-mediated autophagy. However, the role of HIF-1α/BNIP3-mediated autophagy in RFA-induced HCC promotion remains unclear. A recent study conducted by Xu et al. (2019) showed that the proliferation, migration, and invasion abilities of residual HCC cells were significantly elevated after the IRFA was simulated *in vitro*. Compared with the 37°C-si-NC group, the expression of LC3B-II, HIF-1α and BNIP3 were significantly increased in the 47°C-si-NC group, which indicated that IRFA could induce the activation of autophagy and the upregulation of HIF-1α and BNIP3 in HCC cells (Xu et al., 2019). Notably, BNIP3 silencing significantly decreased the expression levels of HIF-1α and LC3B-II as well as slowed the proliferation, migration, and invasion of HCC cells mediated by IRFA (Xu et al., 2019). These studies revealed that IRFA promoted residual HCC cell progression by inducing autophagy *via* the HIF-1α/BNIP3 pathway.

## ATO inhibits tumor growth and angiogenesis of HCC by blocking the paracrine signaling of Ang-1 and Ang-2 through the inhibition of the p-Akt/HIF-1α pathway after IRFA

Angiogenesis is an essential process in the growth and metastasis of HCC (Zhu et al., 2022). Angiogenesis is not only regulated by VEGF but also by angiopoietin-1 (Ang-1) and angiopoietin-2 (Ang-2), and their receptor Tie-2 (Engin et al., 2012). Ang-1 and Ang-2 are the members of the angiopoietin (Ang) family and regulate angiogenesis *via* the TEK tyrosine kinase endothelial receptor (Lin et al., 2020; Xie et al., 2020). Also, Ang-1 and Ang-2 have been shown to be the prognostic biomarkers of HCC(Lin et al., 2020; Xie et al., 2020). Pestana et al. (2018) revealed that a high level of Ang-2 was closely associated with the poor prognosis of HCC patients. In contrast, HCC patients with a high level of Ang-1 showed a longer overall survival (Pestana et al., 2018). Contrary to the studies discussed above, another study found that activated hepatic stellate cells (aHSCs) promoted angiogenesis through secreting Ang-1 (Lin et al., 2020). Wang Z et al. (2021) reported that morphine significantly promoted angiogenesis by activating Akt/HIF-1α pathway in HCC. However, whether Akt/HIF-1α axis is involved in the progression of residual HCC after RFA by modulating Ang-1 or Ang-2 was still unclear. A more recent study indicated that IRFA was simulated using a water bath and promoted tumor growth and angiogenesis of HCC *in vitro* and *in vivo* (Dong et al., 2022). The above phenomenon could be suppressed by arsenic trioxide (ATO) (Dong et al., 2022). Furthermore, higher levels of Ang-1, Ang-2, and p-Tie2 were detected in a conditioned medium from RFA-treated than from untreated HCC cells (Dong et al., 2022). The expression of HIF-1α and p-Akt were also upregulated following RFA in HCC cells (Dong et al., 2022). The levels of Ang-1, Ang-2, p-Tie2, HIF-1α, and p-Akt were suppressed by ATO (Dong et al., 2022). Further studies found that Ang-1 or Ang-2 knockdown impaired the ability of the conditioned medium to promote angiogenesis (Dong et al., 2022). Similar results can be obtained when Tie2 expression was silenced using siRNA (Dong et al., 2022). In addition, YC-1, an inhibitor of HIF-1α, markedly inhibited the increased expression of Ang-1 and Ang-2 in HCC cells (Dong et al., 2022). The levels of Ang-1, Ang-2, p-Tie2, and p-Akt were upregulated following HIF-1α overexpression, and the effect of ATO was attenuated (Dong et al., 2022). These studies indicated that ATO suppressed tumor growth and angiogenesis of HCC by regulating paracrine Ang-1 and Ang-2 secretion through the p-Akt/HIF-1α pathway after IRFA.

## HIF-1α facilitates the progression of HCC by promoting the warburg effect and EMT after IRFA

Continuous aerobic glycolysis has been demonstrated to trigger oncogene development in the cancer cells. Aerobic glycolysis is known as the Warburg effect and is one of the tumor hallmarks (Liu et al., 2022a). The enhanced Warburg effect is closely linked to EMT and

poor patient prognosis in multiple cancer, including HCC. Zhou et al. (2021b) reported that zinc finger E-box-binding homeobox 1 (ZEB1), predicting worse overall survival in cancer patients, facilitated tumorigenesis and metastasis of HCC by enhancing the Warburg effect. It was reported that HIF-1α regulated the Warburg effect by influencing glycolysis, accumulation of lactic acid, and infiltration of the extracellular matrix (Zhou et al., 2022). Lyu et al. (2022) reported that HIF-1α accelerated the Warburg effect and promoted ovarian cancer tumorigenesis by upregulating WT1-associated protein. Liu et al. (2021) demonstrated that by regulating the miR-30c/HIF-1α pathway, FBI-1, an important regulator of HCC, promoted the Warburg effect or EMT of HCC cells and contributed to the drug resistance of HCC cells. A recent study demonstrated that the Warburg effect and level of HIF-1α are enhanced in HCC cells after sublethal heat stress (Chen et al., 2021). 2-NBDG uptake experiment showed that a dramatically increased uptake of glucose was observed in HCC cells after sublethal heat stress (Chen et al., 2021). In addition, HCC cells under normal glucose conditions had a higher survival rates following sublethal heat stress than that under complete glucose-deprivation conditions (Chen et al., 2021). These data indicated that the increased uptake of glucose induced by Warburg effect promoted HCC cell proliferation and invasion (Chen et al., 2021). Further study found that dimethyloxallyl glycine, a specific HIF-1α agonist, improved glucose uptake, and promoted glycolysis-related markers (GLUT-1/3 and LDHA) expression in HCC cells (Chen et al., 2021). Taken together, HIF-1α might facilitate the progression of HCC by regulating the Warburg effect after IRFA.

As known, E-cadherin, N-cadherin, vimentin, and Snail are the markers of EMT (Cessna et al., 2022). Tong et al. (2017) mimicked the IRFA through thermal treatment under hypoxic conditions and found that the protein levels of HIF-1α, N-cadherin, vimentin, Snail, and TGF-β1 following IRFA were significantly elevated, while the expression of E-cadherin was reduced in HCC cells. Moreover, the formation of a hypoxic microenvironment following IRFA significantly promotes HCC cell migration and invasion. Furthermore, N-cadherin, vimentin, Snail, and TGF-β1 expression in shHIF-1α cells were decreased in conjunction with an increased in E-cadherin expression. Previous studies have already demonstrated that TGF-β1 promoted EMT by increasing the expression of vimentin and Snail in HCC (Chen and Yan, 2021; Zhang Z et al., 2021). Of note, upregulation of vimentin and Snail induced by IRFA could be completely inhibited by pretreatment with SB431542, an inhibitor of the TGF-β receptor. All of these data indicated that HIF-1α promoted EMT by TGF-β1 after IRFA, contributing to HCC invasion and metastasis. Figure 1 shows the potential molecular mechanisms of HIF-1, 2α promote angiogenesis and residual recurrence of HCC after IRFA and HIFU ablation.

## Roles of HIF inhibitors for the treatment of HCC and its prospects

von Hippel-Lindau (VHL) serves as a tumor suppressor gene. Hypoxia enhances phagocytosis in neutrophils, and in neutrophils containing a mutated VHL, increased HIF-1α levels lead to decreased apoptosis and increased bacterial phagocytosis under normoxia conditions. HIF-1α can be induced by hypoxia or mutations of VHL. VHL is required to mediate the degradation of HIF-1α in the ubiquitin-proteasome pathway. It was reported that inactivation of the VHL tumor suppressor gene, which results in pseudohypoxia stabilization of both HIF-1α and HIF-2α, is an initiating genetic event for both hereditary (VHL disease) and sporadic renal cell carcinoma (RCC) (Kaelin, 2007). Genetic and functional studies have supported a protumorigenic role of HIF-2α and a tumor suppressor role for HIF-1α in ccRCC, which has prompted the development of HIF-2α–specific inhibitors (Choueiri and Kaelin, 2020). HIF-1α is believed to play an important role during RCC initiation and is elevated in the earliest preneoplastic lesions in VHL patients (Mandriota et al., 2002).

Now, selective HIF-2α antagonists (PT2385 and PT2399) that inhibit HIF-2 transcriptional activity were identified. PT2399 suppressed tumor growth in most human patient-derived xenografts (PDXs) with greater antitumor activity than that of the tyrosine kinase inhibitor (TKI) sunitinib (Chen J et al., 2016). PT2977 (also known as MK-6482, belzutifan), a PT2385 derivative with an improved pharmacokinetic profile and potency, demonstrated promising single-agent activity in heavily pretreated patients with advanced RCC and VHL-associated non-metastatic ccRCC and was recently approved by the FDA for the treatment of cancers associated with VHL disease (Courtney et al., 2020). Inconsistent results were found in the common isoforms of HIF, HIF-1α, and HIF-2α. A recent study using tissue from 380 patients revealed a significant association of high HIF-2α with increased OS, whereas high HIF-1α was significantly associated with higher-grade tumors and reduced OS in both the univariable and multivariable settings (Cowman et al., 2020). As aforementioned, HIF-1α was considered to be a tumor suppressor. This is inconsistent with the finding that high HIF-1α expression was associated with poor survival in RCC (Minardi et al., 2015). Therefore, the inhibition of HIF-1α should continue to be explored as a therapeutic strategy for RCC.

In the HCC setting, HIF-α inhibitors may also play roles in the treatment of HCC. Clinical data have demonstrated that overexpressed HIF-1α and HIF-2α in HCC patients are reliable markers of a poor prognosis (Dai et al., 2018; Mendez-Blanco et al., 2018). At present, only one clinical study was available on ClinicalTrials.gov and Pubmed. This phase I study evaluated the intravenous infusion effect of the HIF- 1α mRNA antagonist RO7070179 in HCC patients failing to respond to systemic therapy, but this study did not show the results [ClinicalTrials.gov Identifier: NCT02564614].

Of note, the majority of studies are preclinical findings. For example, Salman et al. (2022) showed that HIF-1 and HIF-2 inhibitor 32-134D eradicate HCC in combination with anti-PD1 therapy . LW6 is a drug that inhibits hypoxia by reducing HIF-1α accumulation and gene transcriptional activity. Xu et al. (2022) found that LW6 can promote apoptosis of HCC cells by inhibiting HIF-1α, inhibiting tumor angiogenesis, and downregulating the expression of PD-L1, which is an effective choice for the treatment of HCC. Mu et al. (2021) found that HIF-2α knockdown decreased the expression of downstream c-MYC, suppressed hypoxic cell proliferation, and induced HCC cell apoptosis, whereas HIF-1α knockdown did not. Wu et al. the expression of HIF-2α can be inhibited by sorafenib, which is likely to provide an effective adjunct treatment for patients with HCC following HIFU ablation (Wu et al., 2021).

Based on the above evidence, preclinical studies demonstrated that HIF inhibitors have been proposed as one of the effective treatments for HCC. Further clinical investigations would allow for a better

understanding and help to propose more effective strategies to increase the efficacy of HIF inhibitors treatment for HCC.

## Combination therapies in the management of early and unresectable HCC

There have been many studies indicative of the therapeutic benefit of the combination of anticancer therapies for early and unresectable HCC, such as RFA combined with a chemotherapeutic drug, and thermal ablation combined with transarterial chemoembolization. The combination of local ablation therapies with immunotherapies represents a promising therapeutic strategy for treating HCC. The primary immunotherapeutic strategies include immune checkpoint inhibitors therapy (e.g., PD-1 and PD-L1), cell-based therapies, and tumor vaccine therapy. Though the role of PD-1 inhibitors in the adjuvant setting is still under investigation, the administration of PD-1 blockade in addition to RFA was found to be promising. A previous study reported that PD-1 inhibitor combined with RFA for recurrent HCC resulted in a significantly improved 1-year RFS rate compared to RFA alone (Wang X et al., 2021). A multicenter RCT (Lee et al., 2015) showed that adjuvant immunotherapy with activated cytokine-induced killer cells increased recurrence-free and OS in patients with HCC who underwent curative treatment with RFA. In addition, Ji et al. (2021) showed that RFA and TACE combined with postoperative autologous cytokine-induced killer (CIK) cell immunotherapy reinfusion have significant efficacy in the treatment of primary HCC. A preclinical study demonstrated that PI3Kγ inhibitors could enhance anti-PD-1 therapy for the treatment of residual tumors after IRFA (Liu et al., 2022b). However, though these studies provide a strong rationale for combining RFA and the immunotherapies in the clinical setting, the roles of HIF in this action are extremely scarce.

Advances are currently being made in this area of HCC with ablation treatment. A single-arm phase 2 trial (Qiao et al., 2022) demonstrated that addition of anti-PD-1 adjuvant therapy after TACE combined with ablation significantly prolong the relapse-free survival with controllable safety for HCC patients with high recurrence risk. Repeat hepatectomy was considered to serve as the first choice for solitary small recurrent HCC patients with late recurrence, while MWA should be selected for those with early recurrence (Wang et al., 2022). A randomized controlled phase 2 trial conducted by Radosevic et al. (2022) showed that MWA created larger ablation zones than RFA ($p$ = 0.036). The authors concluded that both MWA and RFA were effectiveness and safety in liver tumors between 1.5 and 4 cm. Suh et al. (2021) revealed that no-touch RFA using twin internally cooled wet electrodes demonstrated significantly lower cumulative local tumor progression rates than conventional RFA for small HCCs. A phase 2 study (Iwata et al., 2021) reported that image-guided proton therapy (IGPT) was a safe and effective treatment for solitary operable or ablation-treatable HCC. Zaitoun et al. (2021) revealed that combined therapy with conventional TACE + MWA is safe, well-tolerated, and more effective than TACE or MWA alone for treatment of HCC with 3–5 cm. Previous study showed that sorafenib combined with TACE and RFA resulted in longer recurrence-free survival and better OS than did TACE-RFA in patients with medium or large HCC (Zhu et al., 2018). However, a subsequent study developed by Bockorny et al. (2022) suggested that priming of sorafenib did not enhance the effect of RFA in intermediate sized HCC. The above studies showed that recent studies continue to explore more effective therapeutic strategies by combining hepatectomy or ablation for treating different staging of HCC (Table 2).

With the advance of screening technology and increased awareness of cancer surveillance, more and more HCC could be detected at early stage, rendering curative therapeutics applicable. The therapeutic strategies of HCC are evolving rapidly. In the 2022 update of BCLC strategy for HCC management, local ablation still plays leading part among the recommended curative treatments for early-stage HCC (Chen S et al., 2022). As compare to the less effective alcohol injection, an increased use of radiofrequency ablation has been found to improve management of intermediate stage patients with HCC (Garuti et al., 2021). Adjuvant therapies (e.g. immunotherapy) have been found to prevent against HCC recurrence after curative treatment, which could significantly improve the prognosis. For the combination with imunotherapy, ablative techniques are gaining more and more attention for their capability of boosting local and systemic immune effects, which makes combination strategy a promising weapon for HCC treatment (Chen M et al., 2022). Besides, tyrosine kinase inhibitors, oncolytic virotherapy, and cancer vaccines, may also boost anti-tumor immunity for HCC. These novel therapeutic strategies show great potential to synergize with ablation in the treatment of primary and metastatic HCC.

It was reported that sorafenib in combination with RFA could improve the treatment of HCC due to sorafenib suppresses cell proliferation and induces apoptosis in hepatoma cells by the HIF-1/VEGFA pathway (Gong et al., 2017). The combination of current sorafenib treatment with gene therapy or inhibitors against HIFs has been documented as promising approach to overcome sorafenib resistance both *in vitro* and *in vivo*. Wu et al. (2021) reported that the synergistic effect of the combination of HIFU with sorafenib therapy inhibited HCC tumor growth when compared with HIFU treatment alone or with no treatment, which was closely related to the decreased HIF-2α, VEGFA. Gong et al. (2017) also found that sorafenib in combination with RFA significantly improved the outcomes of early small HCC, which might be associated with decreased serum levels of active tumor growth factors HIF-1α. In addition, a combination of sorafenib and HIFs-targeted therapy or HIFs inhibitors can overcome HCC sorafenib resistance (Zeng et al., 2021). Besides, chloroquine significantly increased the apoptosis of HCC cells after IRFA and inhibited the enrichment of CSCs, and this effect was significantly enhanced by the combination of C-MET inhibitors (Zhao et al., 2018). Except for ablation, HIF inhibitors also play a promising role in treating early or unresectable HCC. For example, HIF inhibitor 32-134D was found to eradicate murine hepatocellular carcinoma in combination with anti-PD1 therapy (Salman et al., 2022). HIF-2α-targeted interventional chemoembolization was detected for the effective elimination of HCC (Chen S et al., 2022).

Taken together, RFA combined with other anti-cancer approaches (i.e. sorafenib, transarterial chemoembolization, and chloroquine) exerts a better curative effect in terms of tumor suppression than RFA alone, probably through inhibiting HIF-1α.

## Conclusion

HIF-1, 2α, the potent factors of tumor angiogenesis, play essential roles in the mechanisms of recurrence of HCC after ablation and are of great significance for the efficacy evaluation of ablation of patients with HCC and the development of individualized treatment options.

HIF-1, 2α targets the different molecules (e.g., BNIP3, CA-IX, and arginase-1) and signaling cascades (e.g., VEGFA/EphA2 pathway) constituting a complex network that promotes HCC invasion and metastasis after ablation. Currently, the inhibitors of HIF have been developed, providing important proofof targeting HIF for the prevention of HCC recurrence after ablation. Though the effects of HIF-1, 2α on HCC after ablation have been preliminarily elucidated, the dynamic changes in HIF expression after ablation of HCC patients remain to be further studied.

## Author contributions

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

## Funding

This project was supported by the grants from Zhejiang Province Public Welfare Technology Application Research Project (No. LGF21H160022), the Natural Science Foundation of Zhejiang Province (No. LQ22H160055), Science and Technology Plan Project of Taizhou (No. 21ywb26, 21ywb29, and 22ywa14), Medical Science and Technology Project of Zhejiang Province (No. 2017KY711, 2023KY403, 2023KY404, and 2023KY1340).

## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

## Publisher's note



## References

Alexander, R. K., Liou, Y. H., Knudsen, N. H., Starost, K. A., Xu, C., Hyde, A. L., et al. (2020). Bmal1 integrates mitochondrial metabolism and macrophage activation. *eLife* 9, e54090. doi:10.7554/eLife.54090

Barquilla, A., Lamberto, I., Noberini, R., Heynen-Genel, S., Brill, L. M., and Pasquale, E. B. (2016). Protein kinase A can block EphA2 receptor-mediated cell repulsion by increasing EphA2 S897 phosphorylation. *Mol. Biol. Cell.* 27 (17), 2757–2770. doi:10.1091/mbc.E16-01-0048

Ben, D. C., Mandi, K., Frye, M., Acheampong, A., Radi, A., De Becker, B., et al. (2022). Chemerin regulates normal angiogenesis and hypoxia-driven neovascularization. *Angiogenesis* 25 (2), 159–179. doi:10.1007/s10456-021-09818-1

Benej, M., Svastova, E., Banova, R., Kopacek, J., Gibadulinova, A., Kery, M., et al. (2020). CA IX stabilizes intracellular pH to maintain metabolic reprogramming and proliferation in hypoxia. *Front. Oncol.* 10, 1462. doi:10.3389/fonc.2020.01462

Bockorny, B., Bullock, A. J., Abrams, T. A., Faintuch, S., Alsop, D. C., Goldberg, S. N., et al. (2022). Priming of sorafenib prior to radiofrequency ablation does not increase treatment effect in hepatocellular carcinoma. *Dig. Dis. Sci.* 67 (7), 3455–3463. doi:10.1007/s10620-021-07156-2

Calderaro, J., Seraphin, T. P., Luedde, T., and Simon, T. G. (2022). Artificial intelligence for the prevention and clinical management of hepatocellular carcinoma. *J. Hepatol.* 76 (6), 1348–1361. doi:10.1016/j.jhep.2022.01.014

Cessna, H., Baritaki, S., Zaravinos, A., and Bonavida, B. (2022). The role of RKIP in the regulation of EMT in the tumor microenvironment. *Cancers (Basel)* 14 (19), 4596. doi:10.3390/cancers14194596

Chen, J., Bai, M., Ning, C., Xie, B., Zhang, J., Liao, H., et al. (2016). Gankyrin facilitates follicle-stimulating hormone-driven ovarian cancer cell proliferation through the PI3K/AKT/HIF-1α/cyclin D1 pathway. *Oncogene* 35 (19), 2506–2517. doi:10.1038/onc.2015.316

Chen, M., Shu, G., Lv, X., Xu, X., Lu, C., Qiao, E., et al. (2022). HIF-2α-targeted interventional chemoembolization multifunctional microspheres for effective elimination of hepatocellular carcinoma. *Biomaterials* 284, 121512. doi:10.1016/j.biomaterials.2022.121512

Chen, S., Zeng, X., Su, T., Xiao, H., Lin, M., Peng, Z., et al. (2022). Combinatory local ablation and immunotherapies for hepatocellular carcinoma: Rationale, efficacy, and perspective. *Front. Immunol.* 13, 1033000. doi:10.3389/fimmu.2022.1033000

Chen, W., Hill, H., Christie, A., Kim, M. S., Holloman, E., Pavia-Jimenez, A., et al. (2016). Targeting renal cell carcinoma with a HIF-2 antagonist. *Nature* 539 (7627), 112–117. doi:10.1038/nature19796

Chen, X., and Yan, N. (2021). Stachydrine inhibits TGF-β1-induced epithelial-mesenchymal transition in hepatocellular carcinoma cells through the TGF-β/Smad and PI3K/Akt/mTOR signaling pathways. *Anticancer Drugs* 32 (8), 786–792. doi:10.1097/CAD.0000000000001066

Chen, Y., Bei, J., Liu, M., Huang, J., Xie, L., Huang, W., et al. (2021). Sublethal heat stress-induced O-GlcNAcylation coordinates the Warburg effect to promote hepatocellular carcinoma recurrence and metastasis after thermal ablation. *Cancer Lett.* 518, 23–34. doi:10.1016/j.canlet.2021.06.001

Chen, Z., Ai, L., Mboge, M. Y., Tu, C., Mckenna, R., Brown, K. D., et al. (2018). Differential expression and function of caix and caxii in breast cancer: A comparison between tumorgraft models and cells. *PLoS One* 13 (7), e0199476. doi:10.1371/journal.pone.0199476

Cheng, N., Brantley, D. M., Liu, H., Lin, Q., Enriquez, M., Gale, N., et al. (2002). Blockade of EphA receptor tyrosine kinase activation inhibits vascular endothelial cell growth factor-induced angiogenesis. *Mol. Cancer Res.* 1 (1), 2–11.

Cho, E. J., Yu, S. J., Kim, K., Cho, H., Cho, Y. Y., Lee, Y. B., et al. (2019). Carbonic anhydrase-IX inhibition enhances the efficacy of hexokinase II inhibitor for hepatocellular carcinoma in a murine model. *J. Bioenerg. Biomembr.* 51 (2), 121–129. doi:10.1007/s10863-019-09788-6

Choueiri, T. K., and Kaelin, W. J. (2020). Targeting the HIF2-VEGF axis in renal cell carcinoma. *Nat. Med.* 26 (10), 1519–1530. doi:10.1038/s41591-020-1093-z

Courtney, K. D., Ma, Y., Diaz, D. L. A., Christie, A., Xie, Z., Woolford, L., et al. (2020). HIF-2 complex dissociation, target inhibition, and acquired resistance with PT2385, a first-in-class HIF-2 inhibitor, in patients with clear cell renal cell carcinoma. *Clin. Cancer Res.* 26 (4), 793–803. doi:10.1158/1078-0432.CCR-19-1459

Cowman, S. J., Fuja, D. G., Liu, X. D., Tidwell, R., Kandula, N., Sirohi, D., et al. (2020). Macrophage HIF-1α is an independent prognostic indicator in kidney cancer. *Clin. Cancer Res.* 26 (18), 4970–4982. doi:10.1158/1078-0432.CCR-19-3890

Cziraki, A., Lenkey, Z., Sulyok, E., Szokodi, I., and Koller, A. (2020). L-Arginine-Nitric oxide-asymmetric dimethylarginine pathway and the coronary circulation: Translation of basic science results to clinical practice. *Front. Pharmacol.* 11, 569914. doi:10.3389/fphar.2020.569914

D'Ignazio, L., Batie, M., and Rocha, S. (2017). Hypoxia and inflammation in cancer, focus on HIF and NF-κB. *Biomedicines* 5 (2), 21. doi:10.3390/biomedicines5020021

Dai, X., Pi, G., Yang, S. L., Chen, G. G., Liu, L. P., and Dong, H. H. (2018). Association of PD-L1 and HIF-1α coexpression with poor prognosis in hepatocellular carcinoma. *Transl. Oncol.* 11 (2), 559–566. doi:10.1016/j.tranon.2018.02.014

De Francesco, E. M., Maggiolini, M., and Musti, A. M. (2018). Crosstalk between notch, HIF-1α and GPER in breast cancer EMT. *Int. J. Mol. Sci.* 19 (7), 2011. doi:10.3390/ijms19072011

Dong, S., Li, Z., Kong, J., Wu, S., Gao, J., and Sun, W. (2022). Arsenic trioxide inhibits angiogenesis of hepatocellular carcinoma after insufficient radiofrequency ablation via blocking paracrine angiopoietin-1 and angiopoietin-2. *Int. J. Hyperth.* 39 (1), 888–896. doi:10.1080/02656736.2022.2093995

Engin, H., Ustundag, Y., Ozel, T. I., and Gokmen, A. (2012). Plasma concentrations of ang-1, ang-2 and tie-2 in gastric cancer. *Eur. Cytokine Netw.* 23 (1), 21–24. doi:10.1684/ecn.2012.0301

Feng, G. W., Dong, L. D., Shang, W. J., Pang, X. L., Li, J. F., Liu, L., et al. (2014). HDAC5 promotes cell proliferation in human hepatocellular carcinoma by up-regulating Six1 expression. *Eur. Rev. Med. Pharmacol. Sci.* 18 (6), 811–816.

Feng, J., Dai, W., Mao, Y., Wu, L., Li, J., Chen, K., et al. (2020). Simvastatin re-sensitizes hepatocellular carcinoma cells to sorafenib by inhibiting HIF-1α/PPAR-γ/PKM2-mediated glycolysis. *J. Exp. Clin. Cancer Res.* 39 (1), 24. doi:10.1186/s13046-020-1528-x

Fitzpatrick, S. F. (2019). Immunometabolism and sepsis: A role for HIF? *Front. Mol. Biosci.* 6, 85. doi:10.3389/fmolb.2019.00085

Frenzel, T., Siekmann, J., Grohmann, C., Valentiner, U., Schmitz, R., Riecken, K., et al. (2018). Locally ablative radiation therapy of a primary human small cell lung cancer tumor decreases the number of spontaneous metastases in two xenograft models. *Int. J. Radiat. Oncol. Biol. Phys.* 100 (4), 1044–1056. doi:10.1016/j.ijrobp.2017.11.044

Fujita, N., Markova, D., Anderson, D. G., Chiba, K., Toyama, Y., Shapiro, I. M., et al. (2012). Expression of prolyl hydroxylases (PHDs) is selectively controlled by HIF-1 and HIF-2 proteins in nucleus pulposus cells of the intervertebral disc: Distinct roles of PHD2 and PHD3 proteins in controlling HIF-1α activity in hypoxia. *J. Biol. Chem.* 287 (20), 16975–16986. doi:10.1074/jbc.M111.334466

Galan-Cobo, A., Ramirez-Lorca, R., Serna, A., and Echevarria, M. (2015). Overexpression of AQP3 modifies the cell cycle and the proliferation rate of mammalian cells in culture. *PLoS One* 10 (9), e0137692. doi:10.1371/journal.pone.0137692

Garuti, F., Neri, A., Avanzato, F., Gramenzi, A., Rampoldi, D., Rucci, P., et al. (2021). The changing scenario of hepatocellular carcinoma in Italy: An update. *Liver Int.* 41 (3), 585–597. doi:10.1111/liv.14735

Gong, J., Zhou, S., and Yang, S. (2019). Vanillic acid suppresses HIF-1α expression via inhibition of mTOR/p70S6K/4E-BP1 and raf/MEK/ERK pathways in human colon cancer HCT116 cells. *Int. J. Mol. Sci.* 20 (3), 465. doi:10.3390/ijms20030465

Gong, Q., Qin, Z., and Hou, F. (2017). Improved treatment of early small hepatocellular carcinoma using sorafenib in combination with radiofrequency ablation. *Oncol. Lett.* 14 (6), 7045–7048. doi:10.3892/ol.2017.7174

Guo, S., Zhang, D., Lu, X., Zhang, Q., Gu, R., Sun, B., et al. (2021). Hypoxia and its possible relationship with endometrial receptivity in adenomyosis: A preliminary study. *Reprod. Biol. Endocrinol.* 19 (1), 7. doi:10.1186/s12958-020-00692-y

Halpern, A. L., Fitz, J. G., Fujiwara, Y., Yi, J., Anderson, A. L., Zhu, Y., et al. (2021). Hepatic thermal injury promotes colorectal cancer engraftment in C57/black 6 mice. *Am. J. Physiol. Cell. Physiol.* 320 (1), C142–C151. doi:10.1152/ajpcell.00071.2020

Han, Z., Liu, D., Chen, L., He, Y., Tian, X., Qi, L., et al. (2021). PNO1 regulates autophagy and apoptosis of hepatocellular carcinoma via the MAPK signaling pathway. *Cell. Death Dis.* 12 (6), 552. doi:10.1038/s41419-021-03837-y

Hu, W., Zheng, S., Guo, H., Dai, B., Ni, J., Shi, Y., et al. (2021). PLAGL2-EGFR-HIF-1/2α signaling loop promotes HCC progression and Erlotinib insensitivity. *Hepatology* 73 (2), 674–691. doi:10.1002/hep.31293

Huang, J., Yan, L., Cheng, Z., Wu, H., Du, L., Wang, J., et al. (2010). A randomized trial comparing radiofrequency ablation and surgical resection for HCC conforming to the Milan criteria. *Ann. Surg.* 252 (6), 903–912. doi:10.1097/SLA.0b013e3181efc656

Ikemoto, T., Shimada, M., and Yamada, S. (2017). Pathophysiology of recurrent hepatocellular carcinoma after radiofrequency ablation. *Hepatol. Res.* 47 (1), 23–30. doi:10.1111/hepr.12705

Iwata, H., Ogino, H., Hattori, Y., Nakajima, K., Nomura, K., Hashimoto, S., et al. (2021). A phase 2 study of image-guided proton therapy for operable or ablation-treatable primary hepatocellular carcinoma. *Int. J. Radiat. Oncol. Biol. Phys.* 111 (1), 117–126. doi:10.1016/j.ijrobp.2021.03.049

Ji, Q., Fu, Y., Zhu, X., Wang, L., and Ling, C. (2021). Effect of RFA and TACE combined with postoperative cytokine-induced killer cell immunotherapy in primary hepatocellular carcinoma. *J. BUON.* 26 (1), 235–242.

Jing, X., Zhou, Y., Ding, J., Wang, Y., Qin, Z., Wang, Y., et al. (2020). The learning curve for thermal ablation of liver cancers: 4,363-Session experience for a single central in 18 years. *Front. Oncol.* 10, 540239. doi:10.3389/fonc.2020.540239

Kaelin, W. J., and Ratcliffe, P. J. (2008). Oxygen sensing by metazoans: The central role of the HIF hydroxylase pathway. *Mol. Cell.* 30 (4), 393–402. doi:10.1016/j.molcel.2008.04.009

Kaelin, W. J. (2007). The von Hippel-Lindau tumor suppressor protein and clear cell renal carcinoma. *Clin. Cancer Res.* 13, 680s–684s. doi:10.1158/1078-0432.CCR-06-1865

Kling, L., Schreiber, A., Eckardt, K. U., and Kettritz, R. (2021). Hypoxia-inducible factors not only regulate but also are myeloid-cell treatment targets. *J. Leukoc. Biol.* 110 (1), 61–75. doi:10.1002/JLB.4RI0820-535R

Kong, J., Kong, J., Pan, B., Ke, S., Dong, S., Li, X., et al. (2012). Insufficient radiofrequency ablation promotes angiogenesis of residual hepatocellular carcinoma via HIF-1α/VEGFA. *PLoS One* 7 (5), e37266. doi:10.1371/journal.pone.0037266

Labib, O. H., Harb, O. A., Khalil, O. H., Baiomy, T. A., Gertallah, L. M., and Ahmed, R. Z. (2020). The diagnostic value of arginase-1, FTCD, and MOC-31 expression in early detection of hepatocellular carcinoma (HCC) and in differentiation between HCC and metastatic adenocarcinoma to the liver. *J. Gastrointest. Cancer* 51 (1), 88–101. doi:10.1007/s12029-019-00211-2

Lal, S., Shekher, A., PuneetNarula, A. S., Abrahamse, H., and Gupta, S. C. (2021). Cannabis and its constituents for cancer: History, biogenesis, chemistry and pharmacological activities. *Pharmacol. Res.* 163, 105302. doi:10.1016/j.phrs.2020.105302

Lee, J. H., Lee, J. H., Lim, Y. S., Yeon, J. E., Song, T. J., Yu, S. J., et al. (2015). Adjuvant immunotherapy with autologous cytokine-induced killer cells for hepatocellular carcinoma. *Gastroenterology* 148 (7), 1383–1391. doi:10.1053/j.gastro.2015.02.055

Li, G., Kong, J., Dong, S., Niu, H., Wu, S., and Sun, W. (2022). Circular BANP knockdown inhibits the malignant progression of residual hepatocellular carcinoma after insufficient radiofrequency ablation. *Chin. Med. J. Engl.* Publish Ahead of Print. doi:10.1097/CM9.0000000000001822

Lin, J., Cao, S., Wang, Y., Hu, Y., Liu, H., Li, J., et al. (2018). Long non-coding RNA UBE2CP3 enhances HCC cell secretion of VEGFA and promotes angiogenesis by activating ERK1/2/HIF-1α/VEGFA signalling in hepatocellular carcinoma. *J. Exp. Clin. Cancer Res.* 37 (1), 113. doi:10.1186/s13046-018-0727-1

Lin, N., Meng, L., Lin, J., Chen, S., Zhang, P., Chen, Q., et al. (2020). Activated hepatic stellate cells promote angiogenesis in hepatocellular carcinoma by secreting angiopoietin-1. *J. Cell. Biochem.* 121 (2), 1441–1451. doi:10.1002/jcb.29380

Liu, J., Yang, C., Huang, X. M., Lv, P. P., Yang, Y. K., Zhao, J. N., et al. (2021). Knockdown of FBI-1 inhibits the Warburg effect and enhances the sensitivity of hepatocellular carcinoma cells to molecular targeted agents via miR-3692/HIF-1α. *Front. Oncol.* 11, 796839. doi:10.3389/fonc.2021.796839

Liu, S. J., Wang, J. Y., Peng, S. H., Li, T., Ning, X. H., Hong, B. A., et al. (2018). Genotype and phenotype correlation in von Hippel-Lindau disease based on alteration of the HIF-alpha binding site in VHL protein. *Genet. Med.* 20 (10), 1266–1273. doi:10.1038/gim.2017.261

Liu, X., Liu, H., Zeng, L., and Lv, Y. (2022a). BRCA1 overexpression attenuates breast cancer cell growth and migration by regulating the pyruvate kinase M2-mediated Warburg effect via the PI3K/AKT signaling pathway. *PeerJ* 10, e14052. doi:10.7717/peerj.14052

Liu, X., Zhang, W., Xu, Y., Xu, X., Jiang, Q., Ruan, J., et al. (2022b). Targeting PI3Kγ/AKT pathway remodels LC3-associated phagocytosis induced immunosuppression after radiofrequency ablation. . 9 (7), e2102182. doi:10.1002/advs.202102182

Lyu, Y., Zhang, Y., Wang, Y., Luo, Y., Ding, H., Li, P., et al. (2022). HIF-1α regulated WTAP overexpression promoting the Warburg effect of ovarian cancer by m6A-dependent manner. *J. Immunol. Res.* 2022, 6130806. doi:10.1155/2022/6130806

Macedo-Silva, C., Miranda-Goncalves, V., Lameirinhas, A., Lencart, J., Pereira, A., Lobo, J., et al. (2020). JmjC-KDMs KDM3A and KDM6B modulate radioresistance under hypoxic conditions in esophageal squamous cell carcinoma. *Cell. Death Dis.* 11 (12), 1068. doi:10.1038/s41419-020-03279-y

Mandriota, S. J., Turner, K. J., Davies, D. R., Murray, P. G., Morgan, N. V., Sowter, H. M., et al. (2002). HIF activation identifies early lesions in VHL kidneys: Evidence for site-specific tumor suppressor function in the nephron. *Cancer Cell.* 1 (5), 459–468. doi:10.1016/s1535-6108(02)00071-5

Mendez-Blanco, C., Fondevila, F., Garcia-Palomo, A., Gonzalez-Gallego, J., and Mauriz, J. L. (2018). Sorafenib resistance in hepatocarcinoma: Role of hypoxia-inducible factors. *Exp. Mol. Med.* 50 (10), 1–9. doi:10.1038/s12276-018-0159-1

Minardi, D., Lucarini, G., Santoni, M., Mazzucchelli, R., Burattini, L., Conti, A., et al. (2015). Survival in patients with clear cell renal cell carcinoma is predicted by HIF-1α expression. *Anticancer Res.* 35 (1), 433–438.

Morimoto, M., Numata, K., Kondou, M., Nozaki, A., Morita, S., and Tanaka, K. (2010). Midterm outcomes in patients with intermediate-sized hepatocellular carcinoma: A randomized controlled trial for determining the efficacy of radiofrequency ablation combined with transcatheter arterial chemoembolization. *Cancer-Am. Cancer Soc.* 116 (23), 5452–5460. doi:10.1002/cncr.25314

Moudi, B., Mahmoudzadeh-Sagheb, H., and Heidari, Z. (2020). Hepatocyte paraffin 1 and arginase-1 are effective panel of markers in HBV-related HCC diagnosis in fine-needle aspiration specimens. *BMC Res. Notes* 13 (1), 388. doi:10.1186/s13104-020-05230-y

Mu, H., Yu, G., Li, H., Wang, M., Cui, Y., Zhang, T., et al. (2021). Mild chronic hypoxia-induced HIF-2α interacts with c-MYC through competition with HIF-1α to induce hepatocellular carcinoma cell proliferation. . 44 (5), 1151–1166. doi:10.1007/s13402-021-00625-w

Muz, B., de la Puente, P., Azab, F., and Azab, A. K. (2015). The role of hypoxia in cancer progression, angiogenesis, metastasis, and resistance to therapy. 3, 83–92. doi:10.2147/HP.S93413

Nguyen, T. H., Conotte, S., Belayew, A., Decleves, A. E., Legrand, A., and Tassin, A. (2021). Hypoxia and hypoxia-inducible factor signaling in muscular dystrophies: Cause and consequences. *Int. J. Mol. Sci.* 22 (13), 7220. doi:10.3390/ijms22137220

Nijkamp, M. W., van der Bilt, J. D., de Bruijn, M. T., Molenaar, I. Q., Voest, E. E., van Diest, P. J., et al. (2009). Accelerated perinecrotic outgrowth of colorectal liver metastases following radiofrequency ablation is a hypoxia-driven phenomenon. *Ann. Surg.* 249 (5), 814–823. doi:10.1097/SLA.0b013e3181a38ef5

Niu, X., Sun, H., Qiu, F., Liu, J., Yang, T., and Han, W. (2021). miR-10b-5p suppresses the proliferation and invasion of primary hepatic carcinoma cells by downregulating EphA2. *Biomed. Res. Int.* 2021, 1382061. doi:10.1155/2021/1382061

Palazon, A., Tyrakis, P. A., Macias, D., Velica, P., Rundqvist, H., Fitzpatrick, S., et al. (2017). An HIF-1α/VEGF-A Axis in cytotoxic T cells regulates tumor progression. *Cancer Cell.* 32 (5), 669–683. doi:10.1016/j.ccell.2017.10.003

Parikh, N. D., and Pillai, A. (2021). Recent advances in hepatocellular carcinoma treatment. *Clin. Gastroenterol. Hepatol.* 19 (10), 2020–2024. doi:10.1016/j.cgh.2021.05.045

Parks, S. K., and Pouyssegur, J. (2017). Targeting pH regulating proteins for cancer therapy-Progress and limitations. *Semin. Cancer Biol.* 43, 66–73. doi:10.1016/j.semcancer.2017.01.007

Pestana, R. C., Hassan, M. M., Abdel-Wahab, R., Abugabal, Y. I., Girard, L. M., Li, D., et al. (2018). Clinical and prognostic significance of circulating levels of angiopoietin-1 and angiopoietin-2 in hepatocellular carcinoma. *Oncotarget* 9 (102), 37721–37732. doi:10.18632/oncotarget.26507

Porter, E. T., and Porter, F. S. (2018). A strain gauge monitor (SGM) for continuous valve gape measurements in bivalve molluscs in response to laboratory induced diel-cycling hypoxia and pH. *J. Vis. Exp.* 138, 57404. doi:10.3791/57404

Qiao, W., Wang, Q., Hu, C., Zhang, Y., Li, J., Sun, Y., et al. (2022). Interim efficacy and safety of PD-1 inhibitors in preventing recurrence of hepatocellular carcinoma after interventional therapy. *Front. Immunol.* 13, 1019772. doi:10.3389/fimmu.2022.1019772

Radosevic, A., Quesada, R., Serlavos, C., Sanchez, J., Zugazaga, A., Sierra, A., et al. (2022). Microwave versus radiofrequency ablation for the treatment of liver malignancies: A randomized controlled phase 2 trial. *Sci. Rep.* 12 (1), 316. doi:10.1038/s41598-021-03802-x

Rankin, E. B., and Giaccia, A. J. (2016). Hypoxic control of metastasis. *Science* 352 (6282), 175–180. doi:10.1126/science.aaf4405

Ren, H., Liu, N. Y., Song, X. F., Ma, Y. S., and Zhai, X. Y. (2011). A novel specific application of pyruvate protects the mouse retina against white light damage: Differential stabilization of HIF-1α and HIF-2α. *Invest. Ophthalmol. Vis. Sci.* 52 (6), 3112–3118. doi:10.1167/iovs.10-5605

Rocha, S. (2007). Gene regulation under low oxygen: Holding your breath for transcription. *Trends biochem. Sci.* 32 (8), 389–397. doi:10.1016/j.tibs.2007.06.005

Saha, D., Mitra, D., Alam, N., Sen, S., Mustafi, S. M., Majumder, P. K., et al. (2022). Lupeol and Paclitaxel cooperate in hindering hypoxia induced vasculogenic mimicry via suppression of HIF-1α-EphA2-Laminin-5γ2 network in human oral cancer. *J. Cell. Commun. Signal.* doi:10.1007/s12079-022-00693-z

Salman, S., Meyers, D. J., Wicks, E. E., Lee, S. N., Datan, E., Thomas, A. M., et al. (2022). HIF inhibitor 32-134D eradicates murine hepatocellular carcinoma in combination with anti-PD1 therapy. *J. Clin. Invest.* 132 (9), e156774. doi:10.1172/JCI156774

Sauvaget, C., Bazikamwe, S., Lucas, E., Ndayikengurukiye, A., Harerimana, S., and Barango, P. (2022). Evaluation of effectiveness, acceptability and safety of thermal ablation in the treatment of cervical neoplasia in Burundi. *Int. J. Cancer.* 151 (7), 1120–1126. doi:10.1002/ijc.34117

Schito, L., and Semenza, G. L. (2016). Hypoxia-inducible factors: Master regulators of cancer progression. *Trends Cancer* 2 (12), 758–770. doi:10.1016/j.trecan.2016.10.016

Semenza, G. L., Nejfelt, M. K., Chi, S. M., and Antonarakis, S. E. (1991). Hypoxia-inducible nuclear factors bind to an enhancer element located 3' to the human erythropoietin gene. *Proc. Natl. Acad. Sci. U. S. A.* 88 (13), 5680–5684. doi:10.1073/pnas.88.13.5680

Shaida, N., Launchbury, R., Boddy, J. L., Jones, C., Campo, L., Turley, H., et al. (2008). Expression of BNIP3 correlates with hypoxia-inducible factor (HIF)-1alpha, HIF-2alpha and the androgen receptor in prostate cancer and is regulated directly by hypoxia but not androgens in cell lines. *Prostate* 68 (3), 336–343. doi:10.1002/pros.20707

Shin, S. W., Ahn, K. S., Kim, S. W., Kim, T. S., Kim, Y. H., and Kang, K. J. (2021). Liver resection versus local ablation therapies for hepatocellular carcinoma within the milan criteria: A systematic review and meta-analysis. *Ann. Surg.* 273 (4), 656–666. doi:10.1097/SLA.0000000000004350

Snell, C. E., Turley, H., Mcintyre, A., Li, D., Masiero, M., Schofield, C. J., et al. (2014). Proline-hydroxylated hypoxia-inducible factor 1α (HIF-1α) upregulation in human tumours. *PLoS One* 9 (2), e88955. doi:10.1371/journal.pone.0088955

Sofuni, A., Asai, Y., Mukai, S., Yamamoto, K., and Itoi, T. (2022). High-intensity focused ultrasound therapy for pancreatic cancer. *J. Med. Ultrason.* doi:10.1007/s10396-022-01208-4

Sperandio, R. C., Pestana, R. C., Miyamura, B. V., and Kaseb, A. O. (2022). Hepatocellular carcinoma immunotherapy. *Annu. Rev. Med.* 73, 267–278. doi:10.1146/annurev-med-042220-021121

Strowitzki, M. J., Cummins, E. P., and Taylor, C. T. (2019). Protein hydroxylation by hypoxia-inducible factor (HIF) hydroxylases: Unique or ubiquitous? *Cells* 8 (5), 384. doi:10.3390/cells8050384

Suh, Y. S., Choi, J. W., Yoon, J. H., Lee, D. H., Kim, Y. J., Lee, J. H., et al. (2021). No-touch vs. Conventional radiofrequency ablation using twin internally cooled wet electrodes for small hepatocellular carcinomas: A randomized prospective comparative study. *Korean J. Radiol.* 22 (12), 1974–1984. doi:10.3348/kjr.2021.0319

Tong, Y., Yang, H., Xu, X., Ruan, J., Liang, M., Wu, J., et al. (2017). Effect of a hypoxic microenvironment after radiofrequency ablation on residual hepatocellular cell migration and invasion. *Cancer Sci.* 108 (4), 753–762. doi:10.1111/cas.13191

Tsai, Y. M., Wu, K. L., Chang, Y. Y., Chang, W. A., Huang, Y. C., Jian, S. F., et al. (2020). Loss of miR-145-5p causes ceruloplasmin interference with PHD-iron Axis and HIF-2α stabilization in lung adenocarcinoma-mediated angiogenesis. *Int. J. Mol. Sci.* 21 (14), 5081. doi:10.3390/ijms21145081

Vara-Perez, M., Rossi, M., Van den Haute, C., Maes, H., Sassano, M. L., Venkataramani, V., et al. (2021). BNIP3 promotes HIF-1α-driven melanoma growth by curbing intracellular iron homeostasis. *EMBO J.* 40 (10), e106214. doi:10.15252/embj.2020106214

Verma, A. K., Bharti, P. S., Rafat, S., Bhatt, D., Goyal, Y., Pandey, K. K., et al. (2021). Autophagy paradox of cancer: Role, regulation, and duality. *Oxid. Med. Cell. Longev.* 2021, 8832541. doi:10.1155/2021/8832541

Wan, J., Wu, W., Huang, Y., Ge, W., and Liu, S. (2016a). Incomplete radiofrequency ablation accelerates proliferation and angiogenesis of residual lung carcinomas via HSP70/HIF-1α. *Oncol. Rep.* 36 (2), 659–668. doi:10.3892/or.2016.4858

Wan, J., Wu, W., and Zhang, R. (2016b). Local recurrence of small cell lung cancer following radiofrequency ablation is induced by HIF-1α expression in the transition zone. *Oncol. Rep.* 35 (3), 1297–1308. doi:10.3892/or.2015.4541

Wang, G. L., Jiang, B. H., Rue, E. A., and Semenza, G. L. (1995). Hypoxia-inducible factor 1 is a basic-helix-loop-helix-PAS heterodimer regulated by cellular O2 tension. *Proc. Natl. Acad. Sci. U. S. A.* 92 (12), 5510–5514. doi:10.1073/pnas.92.12.5510

Wang, G. L., and Semenza, G. L. (1995). Purification and characterization of hypoxia-inducible factor 1. *J. Biol. Chem.* 270 (3), 1230–1237. doi:10.1074/jbc.270.3.1230

Wang, W. Q., Lv, X., Li, J., Li, J., Wang, J. L., Yuan, T., et al. (2022). Repeat hepatectomy versus microwave ablation for solitary and small (≤3 cm) recurrent hepatocellular carcinoma with early or late recurrence: A propensity score matched study. *Eur. J. Surg. Oncol.* doi:10.1016/j.ejso.2022.12.016

Wang, X., Deng, Q., Feng, K., Chen, S., Jiang, J., Xia, F., et al. (2018). Insufficient radiofrequency ablation promotes hepatocellular carcinoma cell progression via autophagy and the CD133 feedback loop. *Oncol. Rep.* 40 (1), 241–251. doi:10.3892/or.2018.6403

Wang, X, X., Liu, G., Chen, S., Bi, H., Xia, F., Feng, K., et al. (2021). Combination therapy with PD-1 blockade and radiofrequency ablation for recurrent hepatocellular carcinoma: A propensity score matching analysis. *Int. J. Hyperth.* 38 (1), 1519–1528. doi:10.1080/02656736.2021.1991011

Wang, Z, Z., Jiang, L., Wang, J., Chai, Z., and Xiong, W. (2021). Morphine promotes angiogenesis by activating PI3K/Akt/HIF-1α pathway and upregulating VEGF in hepatocellular carcinoma. *J. Gastrointest. Oncol.* 12 (4), 1761–1772. doi:10.21037/jgo-20-394

Wilson, K., Shiuan, E., and Brantley-Sieders, D. M. (2021). Oncogenic functions and therapeutic targeting of EphA2 in cancer. *Oncogene* 40 (14), 2483–2495. doi:10.1038/s41388-021-01714-8

Wu, L., Fu, Z., Zhou, S., Gong, J., Liu, C. A., Qiao, Z., et al. (2014). HIF-1α and HIF-2α: Siblings in promoting angiogenesis of residual hepatocellular carcinoma after high-intensity focused ultrasound ablation. *PLoS One* 9 (2), e88913. doi:10.1371/journal.pone.0088913

Wu, L., Zhang, Y. S., Ye, M. L., Shen, F., Liu, W., Hu, H. S., et al. (2017). Overexpression and correlation of HIF-2α, VEGFA and EphA2 in residual hepatocellular carcinoma following high-intensity focused ultrasound treatment: Implications for tumor recurrence and progression. *Exp. Ther. Med.* 13 (6), 3529–3534. doi:10.3892/etm.2017.4428

Wu, L., Zhou, J., Zhou, W., Huang, X. F., Chen, Q., Wang, W., et al. (2021). Sorafenib blocks the activation of the HIF-2α/VEGFA/EphA2 pathway, and inhibits the rapid growth of residual liver cancer following high-intensity focused ultrasound therapy *in vivo*. *Pathol. Res. Pract.* 220, 153270. doi:10.1016/j.prp.2020.153270

Xie, J. Y., Wei, J. X., Lv, L. H., Han, Q. F., Yang, W. B., Li, G. L., et al. (2020). Angiopoietin-2 induces angiogenesis via exosomes in human hepatocellular carcinoma. *Cell. Commun. Signal* 18 (1), 46. doi:10.1186/s12964-020-00535-8

Xu, H., Chen, Y., Li, Z., Zhang, H., Liu, J., and Han, J. (2022). The hypoxia-inducible factor 1 inhibitor LW6 mediates the HIF-1α/PD-L1 axis and suppresses tumor growth of hepatocellular carcinoma *in vitro* and *in vivo*. *Eur. J. Pharmacol.* 930, 175154. doi:10.1016/j.ejphar.2022.175154

Xu, M., Xie, X. H., Xie, X. Y., Xu, Z. F., Liu, G. J., Zheng, Y. L., et al. (2013). Sorafenib suppresses the rapid progress of hepatocellular carcinoma after insufficient radiofrequency ablation therapy: An experiment *in vivo*. *Acta Radiol.* 54 (2), 199–204. doi:10.1258/ar.2012.120249

Xu, S., Zhou, Z., Peng, X., Tao, X., Zhou, P., Zhang, K., et al. (2021). EBV-LMP1 promotes radioresistance by inducing protective autophagy through BNIP3 in nasopharyngeal carcinoma. *Cell. Death Dis.* 12 (4), 344. doi:10.1038/s41419-021-03639-2

Xu, W. L., Wang, S. H., Sun, W. B., Gao, J., Ding, X. M., Kong, J., et al. (2019). Insufficient radiofrequency ablation-induced autophagy contributes to the rapid progression of residual hepatocellular carcinoma through the HIF-1α/BNIP3 signaling pathway. *BMB Rep.* 52 (4), 277–282. doi:10.5483/BMBRep.2019.52.4.263

Yamada, S., Utsunomiya, T., Morine, Y., Imura, S., Ikemoto, T., Arakawa, Y., et al. (2014). Expressions of hypoxia-inducible factor-1 and epithelial cell adhesion molecule are linked with aggressive local recurrence of hepatocellular carcinoma after radiofrequency ablation therapy. *Ann. Surg. Oncol.* 21, S436–S442. doi:10.1245/s10434-014-3575-z

Yamamoto, K., Venida, A., Yano, J., Biancur, D. E., Kakiuchi, M., Gupta, S., et al. (2020). Autophagy promotes immune evasion of pancreatic cancer by degrading MHC-I. *Nature* 581 (7806), 100–105. doi:10.1038/s41586-020-2229-5

Ye, M., Fang, Z., Gu, H., Song, R., Ye, J., Li, H., et al. (2017). Histone deacetylase 5 promotes the migration and invasion of hepatocellular carcinoma via increasing the transcription of hypoxia-inducible factor-1α under hypoxia condition. *Tumour Biol.* 39 (6), 1010428317705034. doi:10.1177/1010428317705034

You, J., Chen, W., Chen, J., Zheng, Q., Dong, J., and Zhu, Y. (2018). The oncogenic role of ARG1 in progression and metastasis of hepatocellular carcinoma. *Biomed. Res. Int.* 2018, 2109865. doi:10.1155/2018/2109865

Yousaf, M. N., Ehsan, H., Muneeb, A., Wahab, A., Sana, M. K., Neupane, K., et al. (2020). Role of radiofrequency ablation in the management of unresectable pancreatic cancer. *Front. Med. (Lausanne)* 7, 624997. doi:10.3389/fmed.2020.624997

Yuan, G., Zeng, C. L., Zhu, D. D., and Shi, X. J. (2017). Influences of RFA combined with TACE on the HIF-1alpha and EGR level of patients with primary hepatic carcinoma. *Eur. Rev. Med. Pharmacol. Sci.* 21 (8), 1738–1745.

Zaitoun, M., Elsayed, S. B., Zaitoun, N. A., Soliman, R. K., Elmokadem, A. H., Farag, A. A., et al. (2021). Combined therapy with conventional trans-arterial chemoembolization (cTACE) and microwave ablation (MWA) for hepatocellular carcinoma >3-<5 cm. *Int. J. Hyperth.* 38 (1), 248–256. doi:10.1080/02656736.2021.1887941

Zeng, Z., Lu, Q., Liu, Y., Zhao, J., Zhang, Q., Hu, L., et al. (2021). Effect of the hypoxia inducible factor on sorafenib resistance of hepatocellular carcinoma. *Front. Oncol.* 11, 641522. doi:10.3389/fonc.2021.641522

Zhang, H. S., Zhang, Z. G., Du, G. Y., Sun, H. L., Liu, H. Y., Zhou, Z., et al. (2019). Nrf2 promotes breast cancer cell migration via up-regulation of G6PD/HIF-1α/Notch1 axis. *J. Cell. Mol. Med.* 23 (5), 3451–3463. doi:10.1111/jcmm.14241

Zhang, J., Zhang, M., Huang, J., Zhang, G., Li, C., Wang, X., et al. (2021). Development and validation of an autophagy-related gene signature for predicting the prognosis of hepatocellular carcinoma. *Biomed. Res. Int.* 2021, 7771037. doi:10.1155/2021/7771037

Zhang, J., Zhang, Q., Lou, Y., Fu, Q., Chen, Q., Wei, T., et al. (2018). Hypoxia-inducible factor-1α/interleukin-1β signaling enhances hepatoma epithelial-mesenchymal transition through macrophages in a hypoxic-inflammatory microenvironment. *Hepatology* 67 (5), 1872–1889. doi:10.1002/hep.29681

Zhang, K., Fang, T., Shao, Y., and Wu, Y. (2021). TGF-beta-MTA1-SMAD7-SMAD3-SOX4-EZH2 signaling Axis promotes viability, migration, invasion and EMT of hepatocellular carcinoma cells. *Cancer Manag. Res.* 13, 7087–7099. doi:10.2147/CMAR.S297765

Zhang, Q., Lou, Y., Zhang, J., Fu, Q., Wei, T., Sun, X., et al. (2017). Hypoxia-inducible factor-2α promotes tumor progression and has crosstalk with Wnt/β-catenin signaling in pancreatic cancer. *Mol. Cancer* 16 (1), 119. doi:10.1186/s12943-017-0689-5

Zhang, Y., Liu, D., Hu, H., Zhang, P., Xie, R., and Cui, W. (2019). HIF-1α/BNIP3 signaling pathway-induced-autophagy plays protective role during myocardial ischemia-reperfusion injury. *Biomed. Pharmacother.* 120, 109464. doi:10.1016/j.biopha.2019.109464

Zhang, Z., Li, Q., Wang, F., Ma, B., Meng, Y., and Zhang, Q. (2021). Identifying hypoxia characteristics to stratify prognosis and assess the tumor immune microenvironment in renal cell carcinoma. *Front. Genet.* 12, 606816. doi:10.3389/fgene.2021.606816

Zhao, Z., Wu, J., Liu, X., Liang, M., Zhou, X., Ouyang, S., et al. (2018). Insufficient radiofrequency ablation promotes proliferation of residual hepatocellular carcinoma via autophagy. *Cancer Lett.* 421, 73–81. doi:10.1016/j.canlet.2018.02.024

Zhou, L., Zhao, Y., Pan, L. C., Wang, J., Shi, X. J., Du, G. S., et al. (2022). Sirolimus increases the anti-cancer effect of Huai Er by regulating hypoxia inducible factor-1α-mediated glycolysis in hepatocellular carcinoma. *World J. Gastroenterol.* 28 (32), 4600–4619. doi:10.3748/wjg.v28.i32.4600

Zhou, Y., Jin, X., Ma, J., Ding, D., Huang, Z., Sheng, H., et al. (2021a). HDAC5 loss impairs RB repression of pro-oncogenic genes and confers CDK4/6 inhibitor resistance in cancer. *Cancer Res.* 81 (6), 1486–1499. doi:10.1158/0008-5472.CAN-20-2828

Zhou, Y., Lin, F., Wan, T., Chen, A., Wang, H., Jiang, B., et al. (2021b). ZEB1 enhances Warburg effect to facilitate tumorigenesis and metastasis of HCC by transcriptionally activating PFKM. *Theranostics* 11 (12), 5926–5938. doi:10.7150/thno.56490

Zhou, Y., Yamada, N., Tanaka, T., Hori, T., Yokoyama, S., Hayakawa, Y., et al. (2015). Crucial roles of RSK in cell motility by catalysing serine phosphorylation of EphA2. *Nat. Commun.* 6, 7679. doi:10.1038/ncomms8679

Zhu, J., Wu, Y., Yu, Y., Li, Y., Shen, J., and Zhang, R. (2022). MYBL1 induces transcriptional activation of ANGPT2 to promote tumor angiogenesis and confer sorafenib resistance in human hepatocellular carcinoma. *Cell. Death Dis.* 13 (8), 727. doi:10.1038/s41419-022-05180-2

Zhu, K., Huang, J., Lai, L., Huang, W., Cai, M., Zhou, J., et al. (2018). Medium or large hepatocellular carcinoma: Sorafenib combined with transarterial chemoembolization and radiofrequency ablation. *Radiology* 288 (1), 300–307. doi:10.1148/radiol.2018172028

Zhuang, B. W., Li, W., Wang, W., Li, B., Lu, M. D., Kuang, M., et al. (2021). Treatment effect of radiofrequency ablation versus liver transplantation and surgical resection for hepatocellular carcinoma within milan criteria: A population-based study. *Eur. Radiol.* 31 (7), 5379–5389. doi:10.1007/s00330-020-07551-9

Zou, G., Zhang, X., Wang, L., Li, X., Xie, T., Zhao, J., et al. (2020). Herb-sourced emodin inhibits angiogenesis of breast cancer by targeting VEGFA transcription. *Theranostics* 10 (15), 6839–6853. doi:10.7150/thno.43622

frontiers | Frontiers in Immunology

TYPE Mini Review
PUBLISHED 16 February 2023
DOI 10.3389/fimmu.2023.1092401



OPEN ACCESS

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SPECIALTY SECTION
This article was submitted to Cancer Immunity and Immunotherapy, a section of the journal Frontiers in Immunology

RECEIVED 08 November 2022
ACCEPTED 03 February 2023
PUBLISHED 16 February 2023

CITATION
Jiang J, Huang H, Chen R, Lin Y and Ling Q (2023) Immunotherapy for hepatocellular carcinoma recurrence after liver transplantation, can we harness the power of immune checkpoint inhibitors? *Front. Immunol.* 14:1092401. doi: 10.3389/fimmu.2023.1092401



# Immunotherapy for hepatocellular carcinoma recurrence after liver transplantation, can we harness the power of immune checkpoint inhibitors?

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Hepatocellular carcinoma (HCC) is one of the leading causes of cancer-related death globally and liver transplantation (LT) can serve as the best curative treatment option. However, HCC recurrence after LT remains the major obstacle to the long-term survival of recipients. Recently, immune checkpoint inhibitors (ICIs) have revolutionized the treatment of many cancers and provided a new treatment strategy for post-LT HCC recurrence. Evidence has been accumulated with the real-world application of ICIs in patients with post-LT HCC recurrence. Notably, the use of these agents as immunity boosters in recipients treated with immunosuppressors is still controversial. In this review, we summarized the immunotherapy for post-LT HCC recurrence and conducted an efficacy and safety evaluation based on the current experience of ICIs for post-LT HCC recurrence. In addition, we further discussed the potential mechanism of ICIs and immunosuppressive agents in regulating the balance between immune immunosuppression and lasting anti-tumor immunity.

KEYWORDS

**hepatocellular carcinoma, liver transplantation, immune checkpoint inhibitor, immunosuppression, transplant tolerance**

**Abbreviations:** AFP, alpha-fetoprotein; CNIs, calcineurin inhibitors; CR, complete response; CsA, cyclosporine A; CTLA-4, cytotoxic T lymphocyte antigen 4; HCC, hepatocellular carcinoma; ICIs, immune checkpoint inhibitors; IFN-γ, interferon-γ; IL-2, interleukin-2; irAEs, immune-related adverse events; LT, liver transplantation; MMF, mycophenolate mofetil; MPA, mycophenolic acid; mTOR, mammalian target of rapamycin; NFAT, nuclear factor of activated T-cells; PD, progressive disease; PD-1, programmed cell death protein-1; PD-L1, programmed cell death ligand 1; PR, partial response; RFA, radiofrequency ablation; SD, stable disease; TAC, tacrolimus; TACE, trans-arterial chemoembolization; TKIs, tyrosine kinase inhibitors; Tregs, regulatory T cells; TGF-β: transforming growth factor-β; TNF-α, tumor necrosis factor-α.

## Introduction

With almost 906,000 new cases and 830,000 deaths in 2020, liver cancer has become the third leading cause of cancer death worldwide (1). Hepatocellular carcinoma (HCC) is the most common primary liver cancer, accounting for over 75% of cases (2, 3). Nowadays, liver transplantation (LT) for early-stage HCC has become a standard treatment and accounts for nearly 40% of all liver transplantations performed at most centers worldwide (4). Although the prognosis of HCC patients was markedly improved after LT due to the advances in surgical techniques and immunosuppressive agents, HCC recurrence remains the major obstacle to long-term survival.

In the past decades, numerous risk factors have been identified for HCC recurrence, including the pre-transplant alpha-fetoprotein levels, tumor number and size, etc. Therefore, some criteria, such as Milan criteria (5), University of California San Francisco criteria (6) and Hangzhou criteria (7), were advocated to select candidates who might benefit from LT. These strict criteria can minimize the risks, while the HCC recurrence rate after LT is still relatively high, approximately 10% to 30% (4). Several studies reported that the post-LT immunosuppressive environment could be the key hazard factor for HCC recurrence (8, 9), as it could promote tumor escape and cancer cell proliferation by suppressing the proliferation, differentiation and effector functions of T cells (10).

Post-LT HCC recurrence progressed with a predominant pattern of extra-hepatic metastases, including lung, bone and abdominal lymph nodes (4). For the treatment of these tumors, surgical interventions, such as resection (11), trans-arterial chemoembolization (TACE) (12) and radiofrequency ablation (RFA) (13), are meaningful when the nodule is oligo-metastatic and local. For those unresectable nodules, systemic therapy has attracted great attention. Tyrosine kinase inhibitors (TKIs) such as sorafenib and lenvatinib, which are the first-line treatment strategies for advanced HCC, have been applied in recipients with HCC recurrence and proved to be of significant value (14). Sorafenib and lenvatinib can significantly prolong the survival of post-LT patients, and their safety and efficiency have been already evaluated (15, 16). In a meta-analysis, Li Z et al. (15) reviewed 23 studies and concluded that recipients treated with sorafenib for post-LT HCC recurrence had a median survival of 12.8 months and a pooled 1-year survival of 56.8%, better than that observed in patients with the best supportive care. In addition, Chen YY et al. (16) investigated the efficacy of lenvatinib and found a disease control rate of 70%. They also confirmed a comparable efficacy in both LT and non-LT patients in clinical practice. Moreover, several studies have reported the real-world application of immune checkpoint inhibitors (ICIs) in these patients. Different from primary HCC, these relapsed tumors have a higher immune evasion characteristic due to the accumulation of inhibitory cytokines and molecules (17). Single-cell RNA sequencing further revealed that the activation of T cells in recurrent HCC was significantly inhibited by the up-regulation of immune checkpoints (17), suggesting that ICIs-based immunotherapy was promising for the treatment of recurrent HCC in LT recipients. Additionally, patients with recurrent HCC usually have no other way but to try to use the ICIs, due to distant metastasis and TKIs-resistance (18). Notably, while ICIs activate the anti-tumor immunity, they also put grafts in danger of rejection, resulting in limited use thus far. In this review, we appraise the current understanding of the immunotherapy for post-LT HCC recurrence with special attention to the efficacy and safety evaluation based on the current experience of ICIs. We also discussed the potential mechanism underlying the role of ICIs in altering the balance between cancer immunology and transplant tolerance.

## The status of immunosuppressive agents after LT

Currently, various immunosuppressive medications are used in recipients after LT, including steroids, anti-metabolites, mammalian target of rapamycin (mTOR) inhibitors and calcineurin inhibitors (CNIs) (10). Immunosuppressive agents have resulted in decreased incidence of acute rejection and to prolong graft survival of LT recipients, but also cause adverse events (19). CNIs, such as cyclosporine A (CsA) and tacrolimus (TAC), are the cornerstone of immunosuppressive regimens with profound significance in preventing graft rejection. Both TAC and CsA can inhibit the $Ca^{2+}$/Calcineurin/nuclear factor of activated T-cells (NFAT) pathway, reduce the secretion of interleukin-2 (IL-2) and interferon-γ (IFN-γ), and contribute to long-term allograft survival (10). However, studies in human cohorts reported that overexposure to TAC and CsA increased the risk of post-LT HCC recurrence (20, 21). Furthermore, both *in vitro* and *in vivo* studies showed that CNIs could enhance the expression of transforming growth factor-β (TGF-β) and promote the proliferation of cancer cells (22, 23).

Mycophenolate mofetil (MMF) is an anti-metabolite purine antagonist and its application in LT began in the late 1990s (24). Given the lack of nephrotoxicity and neurotoxicity, MMF has been used in CNI- or steroid- sparing regimens. However, it remains controversial whether MMF will increase the risk of HCC recurrence after LT. With clinically achievable concentrations, Chen et al. (25) demonstrated that MPA, the active ingredient of MMF, could effectively inhibit cancer cell proliferation and the growth of liver tumor organoids. In addition, authors also found that the use of MMF in LT recipients was significantly associated with less tumor recurrence and improved patient survival. Notably, the result was reported with low precision due to the small sample size (44 LT patients identified as HCC-related LT were included). While a cohort study in Taiwan showed the opposite conclusion, demonstrating that high-dose MMF notably promoted HCC recurrence and reduced the overall survival of recipients after LT (26). Additionally, as a popular immunosuppressive agent, steroids have been reported to induce the proliferation of cancer cells and increase the risk of HCC recurrence (27). Our previous study demonstrated that recipients with steroids-free immunosuppressive protocol had reduced post-LT HCC recurrence as compared to those with steroids in a human cohort (28).

Nowadays, mTOR inhibitors (rapamycin), such as sirolimus and everolimus, have been reported to be anti-recurrence/metastasis and improve the prognosis of patients who underwent LT for HCC (29). Using mTOR inhibitors as an anti-rejection strategy has been accompanied by numerous studies, and the properties of mTOR complex have been emphasized. By targeting complex 1, the rapamycin could inhibit the thymic T cells proliferation and differentiation (30). Interestingly, considerable evidence showed that

TOR inhibitors could not only prevent allograft rejection (30) but also represent potent anti-cancer effects by directly targeting the cancer cells (31). In a prospective, randomized, open-label, multicenter trial, Geissler EK et al. (32) enrolled 525 patients who underwent LT for HCC and found that broad-based practical incorporation of sirolimus into an immunosuppressive regime could improve outcome in the first 3 to 5 years after LT, while the outcome advantage is eventually lost after 5 years. Subsequently, Schnitzbauer et al. (33) performed a multivariate analysis based on the above trial data and concluded that those patients treated with sirolimus ≥ 3 months had better outcomes, especially in the group with higher alpha-fetoprotein levels. On the other hand, the everolimus-based regimen was also proved to be effective in patients with post-LT HCC recurrence. Patients who had high serum trough levels of everolimus (more than 5 ng/ml) had better survival compared to those treated with less than 5 ng/ml (34). In addition, early introduction of everolimus with reduced-CNIs is also associated with a significant renal benefit compared with CNsI-based immunosuppressive regime (35).

## Immune checkpoint inhibitors

The discovery and clinical implementation of ICI has achieved remarkable clinical outcomes and revolutionized the treatment of cancer, as recognized by the 2018 Nobel Prize for Medicine and Physiology (36). There are three main classes of ICIs approved by FDA for clinical application, the inhibitors of programmed cell death protein-1 (PD-1), programmed cell death ligand 1 (PD-L1) and cytotoxic T lymphocyte antigen 4 (CTLA-4). Despite the promising results with immunotherapy in HCC, the safety of using ICIs for post-LT HCC recurrence remains disputed. Different from immunotherapy for primary HCC, post-LT ICIs treatment must be undertaken with caution due to the risk of allograft rejection or graft loss. Here we include all published 27 cases of LTs with ICI treatment for post-LT HCC recurrence (Table 1). The median patient age was 49.4 (range: 14-70) years and 81.5% were males. The median time from LT to ICIs was 2.7 years. The immunotherapy regimens included PD-1 inhibitors (16 nivolumab, 4 toripalimab, 2 pembrolizumab, 1 camrelizumab), PD-L1 inhibitors (2 atezolizumab), CTLA-4 inhibitor (1 ipilimumab) and combination therapy (1 nivolumab followed by atezolizumab). There were 8 (29.6%) patients with disease control, which was defined by stable disease (SD, n=3), partial response (PR, n=1) and complete response (CR, n=4). Ten (37.0%) patients were found to be progressive disease (PD). Of note, graft rejection was reported in 6 out of 27 patients (22.2%), a much higher rate than in patients without ICIs treatment (53), and all of them were treated with nivolumab. To further evaluate the safety of ICIs in recipients, we next reviewed the records of using ICIs in patients with *de novo* malignancies after LT (Table 2). The median age of these patients was 59.4 (range: 35-72) years and 78.57% were males. Melanoma was the main indication for ICIs therapy (n=7), which is followed by lung cancer (n=2). The median time from LT in this setting was longer than that in those with HCC recurrence (7.3 years versus 2.7 years). Among the liver recipients with *de novo* malignancies, 2 patients achieved CR, 4 patients with PR and 4 patients with PD. The graft rejection rate in this group was 21.4%, similar to that in the post-LT HCC recurrence setting.

Several factors may be related to the risk of acute rejection after ICIs treatment based on the current data. First, we observed the rejection rate was lower in anti-PD-L1 group (0/2) than that in anti-PD-1 (8/32) and anti-CTLA-4 (1/4) groups. However, due to the limited cases, the current evidence is not certain to conclude that anti-PD-L1 therapy is relatively safe for post-LT HCC recurrence. Second, a longer interval from LT to initial ICIs treatment and a lower dose of ICIs might be related to a lower incidence of rejection. We found that patients without graft rejection after ICIs treatment have a longer interval from LT to drug exposure (4.65 yr vs. 2.52 yr), which is consistent with the previous studies (65). In addition, a series of cases demonstrated that patients receiving liver grafts with a high level of PD-L1 were prone to develop graft rejection after ICIs therapies (65, 66). Given that, Shi et al. (50) designed a pilot study to evaluate the rejection risk in liver grafts with different PD-L1 expressions. Among 5 recipients who suffered HCC recurrence and were treated with anti-PD-1 therapy (toripalimab), 4 with PD-L1-negative graft did not have rejection, while the other with PD-L1-positive graft developed rejection (50), suggesting that pathological assessment of the graft's PD-L1 status may serve as a selection criterion to decrease the risk of graft rejection before ICIs treatment. Herein, we summarized the efficiency and side effects based on the existing data in the Table 3. More well-designed preclinical and clinical studies with a large sample are required to determine the fundamental mechanisms of acute rejection after ICIs treatment.

## The potential mechanism of immune checkpoint inhibitors in altering immune microenvironment and interplaying with immunosuppressive agents

As described above, ICIs showed clinical benefits for the treatment of HCC recurrence but increased the risk of transplant rejection (Figure 1). Therefore, we summarized the potential mechanisms of PD-1/PD-L1 and CTLA-4 inhibitors in boosting the anti-tumor immunity and inducing transplant rejection.

Physiologically, the non-parenchymal cells in liver graft, including regulatory T cells (Tregs), macrophages and dendritic cells (DCs), played vital roles in promoting a tolerogenic microenvironment (67). These cells could secrete anti-inflammatory cytokines (e.g., $PGE_2$, IL-10 and TGF-β) and induce the death of cytotoxic T cells through the increased expression of immune checkpoints, such as PD-1 and CTLA-4 (67). Specifically, with these immune checkpoint molecules phosphorylated, the downstream co-stimulatory pathways would be inhibited in various immune cells, dampening the immune response (68–70).

PD-1 is mainly expressed on T cells and acts as a negative regulator of T-cell activation through the PI3K/AKT/mTOR and RAS/MEK/ERK pathway (69). It was reported that blocking the PD-1 pathway could reduce the apoptosis of $CD8^+$ T cells and increase the granzyme B expression by enhancing the mTOR signaling, further activating the immune system (71). Moreover, the administration of PD-1 inhibitors could up-regulate the proliferation marker Ki67, enhance the expression of the transcription factor T-bet and the secretion of IFN-

TABLE 1 Case reports with the application of ICIs in HCC recurrence patients after LT.

| No. | Age | Gender | Malignancy | TFTI | Treatment before ICIs | ICIs | Dose | Duration | IS therapy before ICIs | IS therapy during ICIs | Rejection | Outcome | Ref |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **1** | 41 | M | HCC | 1 yrs | TACE/MWA | Nivolumab | 3 mg/kg/2 wks | 15 cycles | TAC | TAC | NO | PD | (37) |
| **2** | 20 | M | HCC | 4 yrs | Sorafenib/Capecitabine | Nivolumab | – | 2 cycles | Sirolimus | Sirolimus | AMR/ TCMR | - | (38) |
| **3** | 14 | M | HCC | 3 yrs | Gemcitabine/Oxaliplatin | Nivolumab | – | 1 cycle | TAC | TAC | AMR/ TCMR | - | (38) |
| **4** | 70 | M | HCC | 8 yrs | Sorafenib/Capecitabine/ External bean radiation | Pembrolizumab | 3 mg/kg/2 wks | 3 mths | TAC | TAC | NO | PD | (39) |
| **5** | 56 | M | HCC | 5.5 yrs | Sorafenib | Nivolumab | – | – | TAC | – | NO | CR | (40) |
| **6** | 55 | M | HCC | 1.8 yrs | Sorafenib | Nivolumab | – | – | Sirolimus/MMF | – | NO | PD | (40) |
| **7** | 34 | F | HCC | 3.7 yrs | Sorafenib | Nivolumab | – | – | TAC | – | NO | PD | (40) |
| **8** | 63 | M | HCC | 1.2 yrs | Sorafenib | Nivolumab | – | – | TAC | – | NO | - | (40) |
| **9** | 68 | M | HCC | 1.1 yrs | Sorafenib | Nivolumab | – | – | Sirolimus | – | YES | - | (40) |
| **10** | 53 | F | HCC | 3 yrs | Sorafenib | Nivolumab | 200 mg/2 wks | 1 cycle | Prednisone/MMF/ Everolimus | Everolimus/ MMF | TCMR | - | (41) |
| **11** | 61 | M | HCC | 2 yrs | Sorafenib | Nivolumab | – | 1 mth | – | – | TCMR | - | (42) |
| **12** | 57 | M | HCC | 3 yrs | Sorafenib | Pembrolizumab | 200 mg/3 wks | 10 mths | TAC/MMF /Steroid | TAC/ Sirolimus | NO | CR | (43) |
| **13** | 64 | M | HCC | 2 yrs | Sorafenib | Nivolumab | – | 0.25 mths | – | – | TCMR | - | (44) |
| **14** | 70 | M | HCC | 3 yrs | Sorafenib/Gemcitabine/Oxaliplatin | Nivolumab | 240 mg/2 wks | 4 cycles | TAC | TAC | NO | PD | (45) |
| **15** | 62 | F | HCC | 2 yrs | Sorafenib/Regorafenib/5Fluorouracil/ Oxaliplatin | Nivolumab | 240 mg/2 wks | 5 cycles | TAC | TAC | NO | SD | (45) |
| **16** | 66 | M | HCC | 2 yrs | Sorafenib/Regorafenib Gemcitabine/Oxaliplatin | Nivolumab | – | 6 cycles | TAC | TAC | NO | PD | (45) |
| **17** | 62 | F | HCC | 2 yrs | TACE | Nivolumab | – | 16 mths | TAC/MMF | – | NO | CR | (46) |

*(Continued)*





10.3389/fimmu.2023.1092401

TABLE 1 Continued

| No. | Age | Gender | Malignancy | TFTI | Treatment before ICIs | ICIs | Dose | Duration | IS therapy before ICIs | IS therapy during ICIs | Rejection | Outcome | Ref |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **18** | 54 | F | HCC | 7 yrs | Sorafenib/Nanoknife /Ethanol ablation | Ipilimumab | 3 mg/kg/3 wks | 13 mths | Everolimus/TAC | Everolimus /TAC | NO | PR | (47) |
| **19** | 54 | M | HCC | 4 yrs | Sorafenib/RFA /Lenvatinib | Camrelizumab | 200 mg/3 wks | 5 cycles | TAC | Sirolimus | NO | CR | (48) |
| **20** | 54 | M | HCC | 2 yrs | Sorafenib/mFolfox-6/ Gemcitabine/TACE | Nivolumab | 200 mg/2 wks | 12 cycles | TAC | TAC | NO | PD | (49) |
| **21** | 46 | M | HCC | 1 yrs | Sorafenib/Lenvatinib | Toripalimab | 240 mg/3 wks | 6 cycles | Sirolimus | Sirolimus | NO | PD | (50) |
| **22** | 46 | M | HCC | 1 yrs | TACE/PEI/Resection /Sorafenib/Lenvatinib | Toripalimab | 240 mg/3 wks | 2 cycles | Sirolimus | Sirolimus | NO | SD | (50) |
| **23** | 62 | M | HCC | 1 yrs | Sorafenib/Lenvatinib /TACE/PEI | Toripalimab | 240 mg/3 wks | – | Sirolimus | Sirolimus | NO | - | (50) |
| **24** | 66 | M | HCC | 1 yrs | Sorafenib/Lenvatinib /Regorafenib | Toripalimab | 240 mg/3 wks | – | Sirolimus | Sirolimus | NO | - | (50) |
| **25** | 35 | M | HCC | 4 yrs | Surgical/Gemcitabine/Oxaliplatin/ Fluorouracil/ IFN alfa-2b | Atezolizumab | – | 6 mths | – | – | NO | PD | (51) |
| **26** | 53 | M | HCC | – | Sorafenib/Resection/ External radiotherapy | Nivolizumab/ Atezolizumab | – | 7 cycles | – | – | NO | SD | (52) |
| **27** | 55 | M | HCC | 1 yrs | Ablation/TACE/ External radiotherapy | Atezolizumab | | 2 cycles | – | – | NO | PD | (52) |

TFTI, time from transplant to ICIs; ICI, immune checkpoint inhibitor; IS, immunosuppressive; Ref, references; M, male; F, female; HCC, hepatocellular carcinoma; TACE, transarterial chemoembolization; MWA, microwave ablation; TAC, tacrolimus; AMR, antibody-mediated rejection; TCMR, T cell–mediated rejection; IFN, interferon; PEI, percutaneous ethanol injection; MMF, mycophenolate mofetil; RFA, radiofrequency ablation; PD, progressive disease; CR, complete response; SD, stable disease; PR, partial response.





10.3389/fimmu.2023.1092401

TABLE 2 Case reports with the application of ICIs in *de novo* malignancy after LT.

| No. | Age | Gender | Reasons for LT | Malignancy After LT | TFTI | ICIs | Dose | Duration | IS therapy before ICIs | IS therapy during ICIs | Rejection | Outcome | Ref |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **1** | 67 | M | HCC | Melanoma | 8 yrs | Ipilimumab | – | 3 mths | Sirolimus | Sirolimus | NO | PR | (54) |
| **2** | 59 | F | Cirrhosis | Melanoma | 8 yrs | Ipilimumab | – | 3 mths | Tacrolimus | Tacrolimus | NO | PD | (55) |
| **3** | 67 | F | LMFM | Melanoma | 1.5 yrs | Ipilimumab | 3mg/kg | 0.75 mths | Sirolimus/MMF | – | YES | PD | (56) |
| **4** | 35 | M | Biliary atresia | Melanoma | 20 yrs | Pembrolizumab | – | 2 cycles | MMF/Steroid | Steroid | NO | CR | (57) |
| **5** | 54 | M | Cirrhosis | NSCLC | 13 yrs | Nivolumab | 3mg/kg | 3 cycles | Tacrolimus/Everolimus/ Prednisone | Tacrolimus/Everolimus/ Prednisone | NO | PD | (58) |
| **6** | 57 | M | HCC | Melanoma | 5.5 yrs | Pembrolizumab | – | – | MMF/Everolimus | – | NO | CR | (40) |
| **7** | 63 | M | CC | Melanoma | 3.1 yrs | Pembrolizumab | – | – | MMF/Prednisone | – | YES | – | (40) |
| **8** | 62 | F | HCC | MPNST-like melanoma | 6 yrs | Ipilimumab/ Pembrolizumab | – | 4 cycles/ 25 cycles | Prednisone/Tacrolimus | Prednisone | NO | PR | (59) |
| **9** | 61 | M | Cirrhosis | Colon adenocarcinoma | 3 yrs | Pembrolizumab | 200 mg/3 wks | 15 cycles | Tacrolimus/MMF/ Prednisone | Tacrolimus | NO | PR | (60) |
| **10** | 66 | M | Cryptogenic liver disease. | Lung adenocarcinoma | 3 yrs | Nivolumab | 3 mg/kg | 0.5M | – | – | YES | – | (61) |
| **11** | 58 | M | PSC-related liver disease | Cutaneous scc | 21 yrs | Nivolumab/ Cemiplimab | 240 mg/2 wks; 350 mg/3 wks | 15M/2 cycles | Tacrolimus/Prednisone | Tacrolimus/Prednisone/MMF | NO | PR | (62) |
| **12** | 52 | M | Alcoholic liver injuries | Hypopharyngeal cancer | 2.7 yrs | Nivolumab | 240mg/2 wks | 4 cycles | Cyclosporine/MMF | Cyclosporine/MMF | NO | – | (63) |
| **13** | 72 | M | – | MCC | 7 yrs | Nivolumab | 3mg/kg/2 wks | 2 cycles | MMF/Budesonide | MMF/Budesonide | NO | – | (64) |
| **14** | 59 | M | ICC | Recurrent ICC | 1 yrs | Toripalimab | 240 mg/3 wks | 7 cycles | Sirolimus | Sirolimus | NO | PD | (50) |

LT, liver transplantation; TFTI, time from transplant to ICIs; ICI, immune checkpoint inhibitor; IS, immunosuppressive; Ref, references; M, male; F, female; HCC, hepatocellular carcinoma; ICI, immune checkpoint inhibitor; MMF, mycophenolate mofetil; SCC, squamous cell carcinoma; TFTI, time from transplant to ICIs; CC, cholangio carcinoma; LMFM, liver metastases from melanoma; NSCLC, non-small cell lung cancer; PSC, primary sclerosing cholangitis; MCC,merkel cell carcinoma; PD, progressive disease; CR, complete response; PR, partial response.





10.3389/fimmu.2023.1092401

TABLE 3 The efficiency and side effects of each drug based on the existing data.

| Drugs | | efficiency | side effects |
|---|---|---|---|
| mTOR's | | The graft rejection rate in those treated with sirolimus is 22.2% (2/9). | Not mentioned. |
| | | The graft rejection rate in those treated with everolimus is 50.0% (1/2). | |
| TKI's | | 81.5% (22/27) patients use TKI's and most of them change to ICI's due to disease progression. | Proteinuria (44); Nausea, Emesis (41) |
| ICI's | PD-1 inhibitors | 28.5% (4/14) patients with disease control. | Graft rejection; Abnormal liver function (38) |
| | PD-L1 inhibitors | 0% (0/2) patients with disease control. | |
| | CTLA-4 inhibitors | 100% (1/1) patients with disease control. | |
| | combination therapy (PD-1 inhibitors +PD-L1 inhibitors) | 100% (1/1) patients with disease control. | |

mTOR, mammalian target of rapamycin; TKI, Tyrosine kinase inhibitors; ICI, immune checkpoint inhibitors; PD-1, programmed cell death protein-1; PD-L1, programmed cell death ligand 1; CTLA-4, cytotoxic T lymphocyte antigen 4.

γ of cytotoxic $CD8^+$ T cells (72). Those cytotoxic $CD8^+$ T cells could not only eliminate the cancer cells but also lead to acute graft rejection (73, 74). In the absence of PD-1 expression, the cytotoxic $CD8^+$ T cells would differentiate into an effector memory phenotype, further prolong the interaction with $CD11c^+$ cells and cause harm to transplant tolerance significantly (75).

Apart from effector T cells, the regulatory T cells (Tregs) could mediate immune response in the pro-inflammatory microenvironments and maintain tolerance in organ transplant models (76). Differently, the immune checkpoint signaling played a controversial role in regulating Treg induction and maintenance. Up to now, several studies have reported that blockade of the CTLA-4 pathway (such as the downstream signaling molecule PP2A) could activate the mTOR signaling (77) and decrease formation of Tregs (78). However, some studies got opposite results and found that inhibition of either PD-1 or CTLA-4 contributes to the proliferation of Tregs and increase the secretion of anti-inflammatory cytokines (79, 80). We summarize the effect of ICI's on Tregs based on current studies in Table 4, and there certainly need more exhaustive studies to figure out the exact role of immune checkpoints in Tregs.

As the ligands of PD-1, PD-L1 is frequently observed in macrophages, DCs, parenchyma cells as well as cancer cells and was found to induce graft tolerance (89). For instance, PD-L1 expressed on the anti-inflammatory phenotype macrophages (M2) was proved to be related to preventing chronic allograft rejection after LT (67). Specifically, these M2 macrophages could increase the number of $Foxp3^+$ Tregs in the liver grafts, contributing to tolerance induction and further prolonging the survival time of recipients (90). Graft-infiltrating DCs, another potent antigen-presenting cell with high PD-L1 expression, have also been shown to contribute to the maintenance

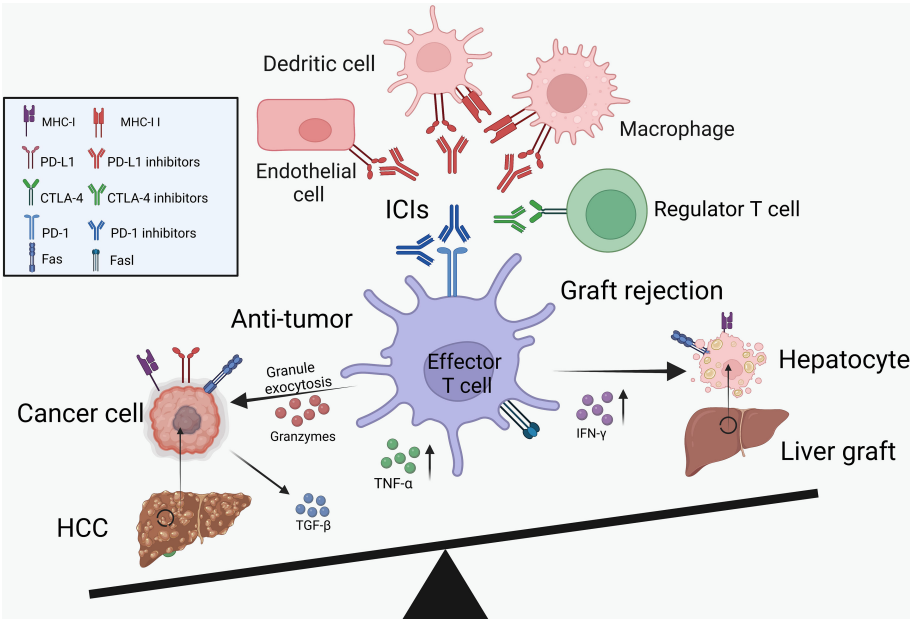


FIGURE 1
The balance between cancer immunology and transplant tolerance. Through the activation of effector T cells, the ICIs can not only reduce tumor burden but also increase the risk of graft rejection. IL-2, interleukin-2; IFN-γ, interferon-γ; TNF-α, tumor necrosis factor-α.

TABLE 4 The effect of each ICI on each cell type.

| Cells | ICIs | Models | Function | reference |
|---|---|---|---|---|
| DCs | PD-L1 inhibitors | MC38 colon cancer model | Activating DC function to enhance T cells killing effect. | (81) |
| | | | Increasing the number of activated (IFN-$\gamma^+$) $CD8^+$ T cells and reactivating tumor-infiltrating T cells. | (82) |
| | | Inflammatory skin reaction | Inhibiting DCs migration from the skin to draining lymph node. | (83) |
| Macrophage | PD-1 inhibitors | MC38 colon cancer mode | Enhancing the capacity for phagocytosis. | (84) |
| | PD-L1 inhibitors | B16 melanoma model | Upregulating mTOR pathway activity and promoting proliferation and survival. | (85) |
| | | MC38 colon cancer model | Inducing T cell activation (more IFN-γ production and higher CD 69 expression). | (81) |
| Tregs | PD-1 inhibitors | Gastric cancer model | promoting the proliferation and immunosuppressive function. | (80) |
| | | Osteosarcoma model | Decreasing the percentage of Tregs in $CD4^+$ T cells. | (86) |
| | CTLA-4 inhibitors | Glycolysis-low tumor model | Enhancing the function of glucose-uptake and IFN-γ production. | (87) |
| | | MC38 colon cancer models | Reducing the number of intra-tumoral Tregs. | (88) |

Tregs, regulatory T cells; DCs, dendritic cells, PD-1, programmed cell death protein-1; PD-L1, programmed cell death ligand 1; CTLA-4, cytotoxic T lymphocyte antigen 4; IFN-γ, interferon-γ.

of graft tolerance (91). These cells could induce the $CD8^+$ T cells exhaustion, subvert anti-donor T cell immune responses and increase the percentage of Tregs (91). However, blockade of the PD-1/PD-L1 interaction by targeting PD-L1 would aggravate the cytotoxic damage caused by $CD8^+$ T cells and enhance the secretion of inflammatory cytokines, such as IL-2, INF-γ and tumor necrosis factor-α (TNF-α) (91). Recently, studies based on the heart and intestinal transplantation models further reported that the blockade or absence of PD-L1 expression on endothelial cells would also result in acute graft rejection by increasing the $CD8^+$ T cells infiltration (92, 93).

We speculate that there could be the following possible reasons. Firstly, PD-L1 is mainly expressed on antigen-presenting cells (including macrophages and DCs) and tumor cells, therefore, PD-L1 antibodies always target these cells, unlike PD-1 antibodies, which directly target T cells to completely block T cell exhaustion. However, macrophages and DCs could also inhibit the activation of T cells by expressing other immune checkpoints, such as TIM-3 and LAG-3 (94, 95). Secondly, the preservation of PD-L2 (another ligand of PD-1) after PD-L1 inhibitor treatment, could partially activate the PD-1 pathway and suppress the immune response, which was proved to be associated with a lower incidence of immune-related adverse events (96). The PD-1 inhibitors could entirely block the interaction between PD-1 and PD-L1/PD-L2, which may lead to T cell over-activation and a higher rejection rate.

To reduce the risk of graft rejection, the combination therapy of ICIs and immunosuppressive agents was proposed, which has attracted great attention recently. Herein, Figure 2 demonstrated the known pathways that control the activation of immune cells and the crosstalk between ICIs and immunosuppressive agents. Recent study revealed that anti PD-1 therapy could activate CD8+ T cells through PI3K-AKT-mTOR pathway and then induces colitis in melanoma patients. Blockade of the pathway with sirolimus not only inhibit tumor growth, but also suppresses the T cell infiltration in colitic lesions, showing a promising strategy for balancing immune overactivation and effective anti-tumor immunity (97). In a kidney transplant case, Esfahani et al. (98) reported that ICI-induced kidney allograft rejection was also associated with cytotoxic CD8+ T cell activation in the periphery, a subset of cells with a well-established role in renal allograft rejection on anti-PD-1 therapy (99). After combination with sirolimus, T cell activation and proliferation was reduced, although IFN-γ-producing CD4+ T cells and cytotoxic CD8 + T cells persisted in circulation. These results further suggested that ICIs and mTOR inhibitors combination therapy promoted a state of functional tolerance without a loss of immune-mediated anti-tumor activity. However, to our knowledge, there are no clinical trials assessing the combination of ICIs and mTOR inhibitors in HCC recurrence after LT. In addition, the protocol of combination therapy still in question. For example, did immunosuppressants need to adjust when combined with ICIs? What is the optimal level of immunosuppressants compared to those without HCC recurrence?

## Conclusion and future expectations

In this review, we summarized the existing research on the immunotherapy of post-LT HCC recurrence and discussed the experience of using ICIs in this setting. We believed that it's better to adopt a steroids-free and mTOR-based regimen in patients with post-LT HCC recurrence instead of the CNIs. Compared to CsA and TAC, sirolimus and everolimus showed a promising role in anti-tumor with mild side effects. Additionally, based on the available data and cases mentioned above, we recommend that physicians should consider cautiously before the application of ICIs. The risks and benefits of ICIs-based immunotherapy must be fully assessed individually, depending on the circumstances of each patient. There are several factors should be taken into account to minimize the risks of graft rejection. Firstly, before the ICIs treatment, negative PD-L1

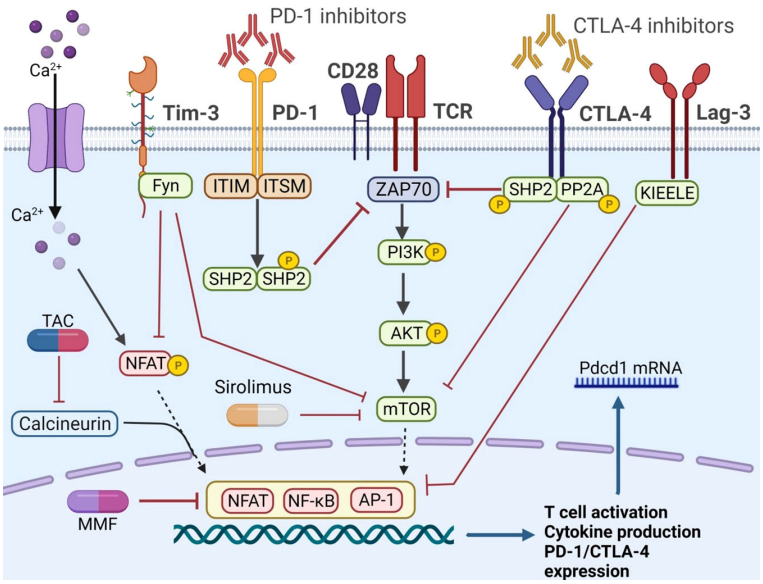


FIGURE 2
The co-stimulatory and co-inhibitory pathways in T cells. The PD-1 axis could phosphorylate ITIM and ITSM, recruit SHP1 and SHP2, and further inhibit ZAP 70. Similarly, CTLA-4 pathway recruited SHP2 and PP2A, and attenuated the mTOR signaling. Fyn is another motif on the cytoplasmic tail of Tim-3, promoting the inhibitory function by inhibiting the NFAT and mTOR activity. The unique KIEELE motif is essential for the inhibitory function of Lag-3. When implemented with ICIs, the co-inhibitory pathway is inhibited and T cell is activated. Immunosuppressive agents, such as CNIs and mTOR inhibitors, can obstruct T cell activation by different mechanisms. PD-1, programmed cell death protein-1; PD-L1, programmed cell death ligand 1; CTLA-4, cytotoxic T lymphocyte antigen 4; PP2A, protein phosphatase 2A; ITIM, immune-receptor tyrosine based inhibitory motif; ITSM, immune-receptor tyrosine based switch motif; ZAP 70, zeta-chain-associated protein kinase 70; SHP, src homology 2 domain- containing protein tyrosine phosphatase; NFAT, nuclear factor of activated T cells; mTOR, mammalian target of rapamycin; Tim-3, T cell immunoglobulin-3; Lag-3, lymphocyte activation gene-3; TIGIT, T cell immunoglobulin and ITIM domain; TAC, tacrolimus.

expression in liver biopsy and increased length of time from LT may contribute to lowering the risk of rejection. Secondly, compared to PD-1 and CTLA-4 inhibitors, PD-L1 therapy is a promising strategy to reduce the risk of graft rejection in post-LT HCC recurrence. Thirdly, the combination protocol (ICIs plus mTOR inhibitors) is a potential strategy to balance cancer immunology and graft tolerance. Moreover, close monitoring of immune status is mandatory during the ICIs therapies, such as the number of $CD4^+$ and $CD8^+$ T cells and the serum of IFN-γ, which were already proved to be helpful for the prediction of graft rejection in kidney and lung transplantation. Finally, once the acute graft rejection occurred, treatments such as ICIs withdrawal, high high-dose steroids and thymoglobulin should be taken immediately to improve patients' outcomes. Further studies about the mechanism of the crosstalk of ICIs and immunosuppressive agents are necessary to improve the therapeutic effect for post-LT HCC recurrence.

## Author contributions

QL and JJ participated in research design. JJ, HH, and RC participated in the writing of the paper. JJ and YL participated in data analysis. JJ, HH, and QL participated in reviewing and editing the manuscript. All authors contributed to the article and approved the submitted version.

## Funding

This work was supported by grants from the National Natural Science Foundation of China (No. 82171757 and 82011530442), Natural Science Foundation of Zhejiang Province (No. LZ22H030004).

## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

## Publisher's note

# References


1. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin* (2021) 71(3):209–49. doi: 10.3322/caac.21660

2. Yang JD, Hainaut P, Gores GJ, Amadou A, Plymoth A, Roberts LR. A global view of hepatocellular carcinoma: Trends, risk, prevention and management. *Nat Rev Gastroenterol Hepatol* (2019) 16(10):589–604. doi: 10.1038/s41575-019-0186-y

3. Llovet JM, Kelley RK, Villanueva A, Singal AG, Pikarsky E, Roayaie S, et al. Hepatocellular carcinoma. *Nat Rev Dis Primers.* (2021) 7(1):6. doi: 10.1038/s41572-020-00240-3

4. Sapisochin G, Bruix J. Liver transplantation for hepatocellular carcinoma: Outcomes and novel surgical approaches. *Nat Rev Gastroenterol Hepatol* (2017) 14(4):203–17. doi: 10.1038/nrgastro.2016.193

5. Mazzaferro V, Regalia E, Doci R, Andreola S, Pulvirenti A, Bozzetti F, et al. Liver transplantation for the treatment of small hepatocellular carcinomas in patients with cirrhosis. *New Engl J Med* (1996) 334(11):693–9. doi: 10.1056/NEJM199603143341104

6. Yao FY, Ferrell L, Bass NM, Watson JJ, Bacchetti P, Venook A, et al. Liver transplantation for hepatocellular carcinoma: expansion of the tumor size limits does not adversely impact survival. *Hepatology.* (2001) 33(6):1394–403. doi: 10.1053/jhep.2001.24563

7. Zheng SS, Xu X, Wu J, Chen J, Wang WL, Zhang M, et al. Liver transplantation for hepatocellular carcinoma: Hangzhou experiences. *Transplantation.* (2008) 85(12):1726–32. doi: 10.1097/TP.0b013e31816b67e4

8. Vivarelli M, Dazzi A, Zanello M, Cucchetti A, Cescon M, Ravaioli M, et al. Effect of different immunosuppressive schedules on recurrence-free survival after liver transplantation for hepatocellular carcinoma. *Transplantation.* (2010) 89(2):227–31. doi: 10.1097/TP.0b013e3181c3c540

9. Mehta N, Bhangui P, Yao FY, Mazzaferro V, Toso C, Akamatsu N, et al. Liver transplantation for hepatocellular carcinoma. working group report from the ILTS transplant oncology consensus conference. *Transplantation.* (2020) 104(6):1136–42. doi: 10.1097/TP.0000000000003174

10. Pallet N, Fernandez-Ramos AA, Loriot MA. Impact of immunosuppressive drugs on the metabolism of T cells. *Int Rev Cell Mol Biol* (2018) 341:169–200. doi: 10.1016/bs.ircmb.2018.05.009

11. Fernandez-Sevilla E, Allard MA, Selten J, Golse N, Vibert E, Sa Cunha A, et al. Recurrence of hepatocellular carcinoma after liver transplantation: Is there a place for resection? *Liver Transpl* (2017) 23(4):440–7. doi: 10.1002/lt.24742

12. Ko HK, Ko GY, Yoon HK, Sung KB. Tumor response to transcatheter arterial chemoembolization in recurrent hepatocellular carcinoma after living donor liver transplantation. *Korean J Radiol* (2007) 8(4):320–7. doi: 10.3348/kjr.2007.8.4.320

13. Huang J, Yan L, Wu H, Yang J, Liao M, Zeng Y. Is radiofrequency ablation applicable for recurrent hepatocellular carcinoma after liver transplantation? *J Surg Res* (2016) 200(1):122–30. doi: 10.1016/j.jss.2015.07.033

14. Sposito C, Mariani L, Germini A, Flores Reyes M, Bongini M, Grossi G, et al. Comparative efficacy of sorafenib versus best supportive care in recurrent hepatocellular carcinoma after liver transplantation: a case-control study. *J Hepatol* (2013) 59(1):59–66. doi: 10.1016/j.jhep.2013.02.026

15. Li Z, Gao J, Zheng S, Wang Y, Xiang X, Cheng Q, et al. Therapeutic efficacy of sorafenib in patients with hepatocellular carcinoma recurrence after liver transplantation: A systematic review and meta-analysis. *Turk J Gastroenterol* (2021) 32(1):30–41. doi: 10.5152/tjg.2020.19877

16. Chen YY, Chen CL, Lin CC, Wang CC, Liu YW, Li WF, et al. Efficacy and safety of lenvatinib in hepatocellular carcinoma patients with liver transplantation: A case-control study. *Cancers (Basel)* (2021) 13(18):4584. doi: 10.3390/cancers13184584

17. Sun Y, Wu L, Zhong Y, Zhou K, Hou Y, Wang Z, et al. Single-cell landscape of the ecosystem in early-relapse hepatocellular carcinoma. *Cell.* (2021) 184(2):404–21.e16. doi: 10.1016/j.cell.2020.11.041

18. Sangro B, Sarobe P, Hervas-Stubbs S, Melero I. Advances in immunotherapy for hepatocellular carcinoma. *Nat Rev Gastroenterol Hepatol* (2021) 18(8):525–43. doi: 10.1038/s41575-021-00438-0

19. Rodríguez-Perálvarez M, de la Mata M, Burroughs AK. Liver transplantation. *Curr Opin Organ Transplantation.* (2014) 19(3):253–60. doi: 10.1097/MOT.0000000000000069

20. Vivarelli M, Cucchetti A, La Barba G, Ravaioli M, Del Gaudio M, Lauro A, et al. Liver transplantation for hepatocellular carcinoma under calcineurin inhibitors: reassessment of risk factors for tumor recurrence. *Ann Surg* (2008) 248(5):857–62. doi: 10.1097/SLA.0b013e3181896278

21. Rodriguez-Peralvarez M, Tsochatzis E, Naveas MC, Pieri G, Garcia-Caparros C, O'Beirne J, et al. Reduced exposure to calcineurin inhibitors early after liver transplantation prevents recurrence of hepatocellular carcinoma. *J Hepatol* (2013) 59(6):1193–9. doi: 10.1016/j.jhep.2013.07.012

22. Hojo M, Morimoto T, Maluccio M, Asano T, Morimoto K, Lagman M, et al. Cyclosporine induces cancer progression by a cell-autonomous mechanism. *Nature.* (1999) 397(6719):530–4. doi: 10.1038/17401

23. Maluccio M, Sharma V, Lagman M, Vyas S, Yang H, Li B, et al. Tacrolimus enhances transforming growth factor-beta1 expression and promotes tumor progression. *Transplantation.* (2003) 76(3):597–602. doi: 10.1097/01.TP.0000081399.75231.3B

24. Di Maira T, Little EC, Berenguer M. Immunosuppression in liver transplant. *Best Pract Res Clin Gastroenterol* (2020) 46-47:101681. doi: 10.1016/j.bpg.2020.101681

25. Chen K, Sheng J, Ma B, Cao W, Hernanda PY, Liu J, et al. Suppression of hepatocellular carcinoma by mycophenolic acid in experimental models and in patients. *Transplantation.* (2019) 103(5):929–37. doi: 10.1097/TP.0000000000002647

26. Tsai YF, Liu FC, Chen CY, Lin JR, Yu HP. Effect of mycophenolate mofetil therapy on recurrence of hepatocellular carcinoma after liver transplantation: A population-based cohort study. *J Clin Med* (2021) 10(8):1558. doi: 10.3390/jcm10081558

27. Gundisch S, Boeckeler E, Behrends U, Amtmann E, Ehrhardt H, Jeremias I. Glucocorticoids augment survival and proliferation of tumor cells. *Anticancer Res* (2012) 32(10):4251–61.

28. Wei Q, Gao F, Zhuang R, Ling Q, Ke Q, Wu J, et al. A national report from China liver transplant registry: steroid avoidance after liver transplantation for hepatocellular carcinoma. *Chin J Cancer Res* (2017) 29(5):426–37. doi: 10.21147/j.issn.1000-9604.2017.05.07

29. Li BCW, Chiu J, Shing K, Kwok GGW, Tang V, Leung R, et al. The outcomes of systemic treatment in recurrent hepatocellular carcinomas following liver transplants. *Adv Ther* (2021) 38(7):3900–10. doi: 10.1007/s12325-021-01800-z

30. Fantus D, Thomson AW. Evolving perspectives of mTOR complexes in immunity and transplantation. *Am J Transplant.* (2015) 15(4):891–902. doi: 10.1111/ajt.13151

31. Mossmann D, Park S, Hall MN. mTOR signalling and cellular metabolism are mutual determinants in cancer. *Nat Rev Cancer.* (2018) 18(12):744–57. doi: 10.1038/s41568-018-0074-8

32. Geissler EK, Schnitzbauer AA, Zulke C, Lamby PE, Proneth A, Duvoux C, et al. Sirolimus use in liver transplant recipients with hepatocellular carcinoma: A randomized, multicenter, open-label phase 3 trial. *Transplantation.* (2016) 100(1):116–25. doi: 10.1097/TP.0000000000000965

33. Schnitzbauer AA, Filmann N, Adam R, Bachellier P, Bechstein WO, Becker T, et al. mTOR inhibition is most beneficial after liver transplantation for hepatocellular carcinoma in patients with active tumors. *Ann Surg* (2020) 272(5):855–62. doi: 10.1097/SLA.0000000000004280

34. Nitta H, Younès A, El-domiaty N, Karam V, Sobesky R, Vibert E, et al. High trough levels of everolimus combined to sorafenib improve patients survival after hepatocellular carcinoma recurrence in liver transplant recipients. *Transplant Int* (2021) 34(7):1293–305. doi: 10.1111/tri.13897

35. Saliba F, Duvoux C, Gugenheim J, Kamar N, Dharancy S, Salame E, et al. Efficacy and safety of everolimus and mycophenolic acid with early tacrolimus withdrawal after liver transplantation: A multicenter randomized trial. *Am J Transplant.* (2017) 17(7):1843–52. doi: 10.1111/ajt.14212

36. Kraehenbuehl L, Weng CH, Eghbali S, Wolchok JD, Merghoub T. Enhancing immunotherapy in cancer by targeting emerging immunomodulatory pathways. *Nat Rev Clin Oncol* (2022) 19(1):37–50. doi: 10.1038/s41571-021-00552-7

37. De Toni EN, Gerbes AL. Tapering of immunosuppression and sustained treatment with nivolumab in a liver transplant recipient. *Gastroenterology.* (2017) 152(6):1631–3. doi: 10.1053/j.gastro.2017.01.063

38. Friend BD, Venick RS, McDiarmid SV, Zhou X, Naini B, Wang H, et al. Fatal orthotopic liver transplant organ rejection induced by a checkpoint inhibitor in two patients with refractory, metastatic hepatocellular carcinoma. *Pediatr Blood Cancer* (2017) 64(12):e26682. doi: 10.1002/pbc.26682

39. Varkaris A, Lewis DW, Nugent FW. Preserved liver transplant after PD-1 pathway inhibitor for hepatocellular carcinoma. *Am J Gastroenterol* (2017) 112(12):1895–6. doi: 10.1038/ajg.2017.387

40. DeLeon TT, Salomao MA, Aqel BA, Sonbol MB, Yokoda RT, Ali AH, et al. Pilot evaluation of PD-1 inhibition in metastatic cancer patients with a history of liver transplantation: the Mayo clinic experience. *J Gastrointest Oncol* (2018) 9(6):1054–62. doi: 10.21037/jgo.2018.07.05

41. Gassmann D, Weiler S, Mertens JC, Reiner CS, Vrugt B, Nageli M, et al. Liver allograft failure after nivolumab treatment-a case report with systematic literature research. *Transplant Direct.* (2018) 4(8):e376. doi: 10.1097/TXD.0000000000000814

42. Gomez P, Naim A, Zucker K, Wong M. A case of hepatocellular carcinoma (HCC) immunotherapy inducing liver transplant rejection. *Am J Gastroenterology.* (2018) 113:S1347–S. doi: 10.14309/00000434-201810001-02415

43. Rammohan A, Reddy MS, Farouk M, Vargese J, Rela M. Pembrolizumab for metastatic hepatocellular carcinoma following live donor liver transplantation: The silver bullet? *Hepatology* (2018) 67(3):1166–8. doi: 10.1002/hep.29575

44. Kumar S. Nivolumab-induced severe allograft rejection in recurrent post-transplant hepatocellular carcinoma. *Am J Gastroenterology.* (2019) 114:S1251–S2. doi: 10.14309/01.ajg.0000598472.41771.5f

45. Al Jarroudi O, Ulusakarya A, Almohamad W, Afqir S, Morere JF. Anti-programmed cell death protein 1 (PD-1) immunotherapy for metastatic hepatocellular carcinoma after liver transplantation: A report of three cases. *Cureus.* (2020) 12(10):e11150. doi: 10.7759/cureus.11150

46. Amjad W, Kotiah S, Gupta A, Morris M, Liu L, Thuluvath PJ. Successful treatment of disseminated hepatocellular carcinoma after liver transplantation with nivolumab. *J Clin Exp Hepatol* (2020) 10(2):185–7. doi: 10.1016/j.jceh.2019.11.009

47. Pandey A, Cohen DJ. Ipilumumab for hepatocellular cancer in a liver transplant recipient, with durable response, tolerance and without allograft rejection. *Immunotherapy.* (2020) 12(5):287–92. doi: 10.2217/imt-2020-0014

48. Qiu J, Tang W, Du C. Immune checkpoint inhibitors in patients with recurrent hepatocellular carcinoma after liver transplantation: A case report and literature review. *Curr Cancer Drug Targets.* (2020) 20(9):720–7. doi: 10.2174/1568009620666200520084415

49. Zhuang L, Mou HB, Yu LF, Zhu HK, Yang Z, Liao Q, et al. Immune checkpoint inhibitor for hepatocellular carcinoma recurrence after liver transplantation. *Hepatobiliary Pancreat Dis Int* (2020) 19(1):91–3. doi: 10.1016/j.hbpd.2019.09.011

50. Shi GM, Wang J, Huang XW, Huang XY, He YF, Ji Y, et al. Graft programmed death ligand 1 expression as a marker for transplant rejection following anti-programmed death 1 immunotherapy for recurrent liver tumors. *Liver Transpl.* (2021) 27(3):444–9. doi: 10.1002/lt.25887

51. Ben Khaled N, Roessler D, Reiter FP, Seidensticker M, Guba M, De Toni EN. Extending the use of atezolizumab and bevacizumab to a liver transplant recipient: Need for a posttransplant registry. *Liver Transpl.* (2021) 27(6):928–9. doi: 10.1002/lt.26011

52. Yang Z, Sun J, Zhuang L, Mou H, Zheng S. Preliminary evaluation of atezolizumab plus bevacizumab as salvage treatment for recurrent hepatocellular carcinoma after liver transplantation. *Liver Transpl* (2022) 28(5):895–6. doi: 10.1002/lt.26416

53. Mao JX, Guo WY, Guo M, Liu C, Teng F, Ding GS. Acute rejection after liver transplantation is less common, but predicts better prognosis in HBV-related hepatocellular carcinoma patients. *Hepatol Int* (2020) 14(3):347–61. doi: 10.1007/s12072-020-10022-4

54. Morales RE, Shoushtari AN, Walsh MM, Grewal P, Lipson EJ, Carvajal RD. Safety and efficacy of ipilimumab to treat advanced melanoma in the setting of liver transplantation. *J Immunother Cancer.* (2015) 3:22. doi: 10.1186/s40425-015-0066-0

55. Ranganath HA, Panella TJ. Administration of ipilimumab to a liver transplant recipient with unresectable metastatic melanoma. *J Immunother.* (2015) 38(5):211. doi: 10.1097/CJI.0000000000000077

56. Dueland S, Guren TK, Boberg KM, Reims HM, Grzyb K, Aamdal S, et al. Acute liver graft rejection after ipilimumab therapy. *Ann Oncol* (2017) 28(10):2619–20. doi: 10.1093/annonc/mdx281

57. Schvartsman G, Perez K, Sood G, Katkhuda R, Tawbi H. Immune checkpoint inhibitor therapy in a liver transplant recipient with melanoma. *Ann Intern Med* (2017) 167(5):361–2. doi: 10.7326/L17-0187

58. Biondani P, De Martin E, Samuel D. Safety of an anti-PD-1 immune checkpoint inhibitor in a liver transplant recipient. *Ann Oncol* (2018) 29(1):286–7. doi: 10.1093/annonc/mdx548

59. Kuo JC, Lilly LB, Hogg D. Immune checkpoint inhibitor therapy in a liver transplant recipient with a rare subtype of melanoma: a case report and literature review. *Melanoma Res* (2018) 28(1):61–4. doi: 10.1097/CMR.0000000000000410

60. Chen JA, Esteghamat N, Kim EJ, Garcia G, Gong J, Fakih MG, et al. PD-1 blockade in a liver transplant recipient with microsatellite unstable metastatic colorectal cancer and hepatic impairment. *J Natl Compr Canc Netw* (2019) 17(9):1026–30. doi: 10.6004/jnccn.2019.7328

61. Braun M, Fuchs V, Kian W, Roisman L, Peled N, Rosenberg E, et al. Nivolumab induced hepatocanalicular cholestasis and liver rejection in a patient with lung cancer and liver transplant. *J Thorac Oncol* (2020) 15(9):e149–e50. doi: 10.1016/j.jtho.2020.01.026

62. Brumfiel CM, Patel MH, Aqel B, Lehrer M, Patel SH, Seetharam M. Immune checkpoint inhibitor therapy in a liver transplant recipient with autoimmune disease and metastatic cutaneous squamous cell carcinoma. *JAAD Case Rep* (2021) 14:78–81. doi: 10.1016/j.jdcr.2021.05.012

63. Kondo T, Kawachi S, Nakatsugawa M, Takeda A, Kikawada N, Aihara Y, et al. Nivolumab for recurrent/metastatic hypopharyngeal squamous cell carcinoma in a liver transplant recipient. *Auris Nasus Larynx* (2021) 49(4):721–6. doi: 10.1016/j.anl.2021.02.002

64. Delyon J, Zuber J, Dorent R, Poujol-Robert A, Peraldi MN, Anglicheau D, et al. Immune checkpoint inhibitors in transplantation-a case series and comprehensive review of current knowledge. *Transplantation.* (2021) 105(1):67–78. doi: 10.1097/TP.0000000000003292

65. Hu B, Yang XB, Sang XT. Liver graft rejection following immune checkpoint inhibitors treatment: a review. *Med Oncol* (2019) 36(11):94. doi: 10.1007/s12032-019-1316-7

66. Munker S, De Toni EN. Use of checkpoint inhibitors in liver transplant recipients. *United Eur Gastroenterol J* (2018) 6(7):970–3. doi: 10.1177/2050640618774631

67. Thomson AW, Vionnet J, Sanchez-Fueyo A. Understanding, predicting and achieving liver transplant tolerance: from bench to bedside. *Nat Rev Gastroenterol Hepatol* (2020) 17(12):719–39. doi: 10.1038/s41575-020-0334-4

68. Ribas A, Wolchok JD. Cancer immunotherapy using checkpoint blockade. *Science.* (2018) 359(6382):1350–5. doi: 10.1126/science.aar4060

69. Patsoukis N, Wang Q, Strauss L, Boussiotis VA. Revisiting the PD-1 pathway. *Sci Adv* (2020) 6(38):eabd2712. doi: 10.1126/sciadv.abd2712

70. Anderson AC, Joller N, Kuchroo VK. Lag-3, Tim-3, and TIGIT: Co-inhibitory receptors with specialized functions in immune regulation. *Immunity.* (2016) 44(5):989–1004. doi: 10.1016/j.immuni.2016.05.001

71. Ahn E, Araki K, Hashimoto M, Li W, Riley JL, Cheung J, et al. Role of PD-1 during effector CD8 T cell differentiation. *Proc Natl Acad Sci U S A.* (2018) 115(18):4749–54. doi: 10.1073/pnas.1718217115

72. Baas M, Besançon A, Goncalves T, Valette F, Yagita H, Sawitzki B, et al. TGFβ-dependent expression of PD-1 and PD-L1 controls CD8+ T cell anergy in transplant tolerance. *eLife* (2016) 5:e08133. doi: 10.7554/eLife.08133

73. Farhood B, Najafi M, Mortezaee K. CD8(+) cytotoxic T lymphocytes in cancer immunotherapy: A review. *J Cell Physiol* (2019) 234(6):8509–21. doi: 10.1002/jcp.27782

74. Morita M, Fujino M, Jiang G, Kitazawa Y, Xie L, Azuma M, et al. PD-1/B7-H1 interaction contribute to the spontaneous acceptance of mouse liver allograft. *Am J Transplant.* (2010) 10(1):40–6. doi: 10.1111/j.1600-6143.2009.02859.x

75. Takahashi T, Hsiao HM, Tanaka S, Li W, Higashikubo R, Scozzi D, et al. PD-1 expression on CD8(+) T cells regulates their differentiation within lung allografts and is critical for tolerance induction. *Am J Transplant.* (2018) 18(1):216–25. doi: 10.1111/ajt.14437

76. Atif M, Conti F, Gorochov G, Oo YH, Miyara M. Regulatory T cells in solid organ transplantation. *Clin Transl Immunol* (2020) 9(2):e01099. doi: 10.1002/cti2.1099

77. Apostolidis SA, Rodriguez-Rodriguez N, Suarez-Fueyo A, Dioufa N, Ozcan E, Crispin JC, et al. Phosphatase PP2A is requisite for the function of regulatory T cells. *Nat Immunol* (2016) 17(5):556–64. doi: 10.1038/ni.3390

78. Besancon A, Baas M, Goncalves T, Valette F, Waldmann H, Chatenoud L, et al. The induction and maintenance of transplant tolerance engages both regulatory and anergic CD4(+) T cells. *Front Immunol* (2017) 8:218. doi: 10.3389/fimmu.2017.00218

79. Paterson AM, Lovitch SB, Sage PT, Juneja VR, Lee Y, Trombley JD, et al. Deletion of CTLA-4 on regulatory T cells during adulthood leads to resistance to autoimmunity. *J Exp Med* (2015) 212(10):1603–21. doi: 10.1084/jem.20141030

80. Kamada T, Togashi Y, Tay C, Ha D, Sasaki A, Nakamura Y, et al. PD-1(+) regulatory T cells amplified by PD-1 blockade promote hyperprogression of cancer. *Proc Natl Acad Sci U S A.* (2019) 116(20):9999–10008. doi: 10.1073/pnas.1822001116

81. Lin H, Wei S, Hurt EM, Green MD, Zhao L, Vatan L, et al. Host expression of PD-L1 determines efficacy of PD-L1 pathway blockade-mediated tumor regression. *J Clin Invest.* (2018) 128(2):805–15. doi: 10.1172/JCI96113

82. Peng Q, Qiu X, Zhang Z, Zhang S, Zhang Y, Liang Y, et al. PD-L1 on dendritic cells attenuates T cell activation and regulates response to immune checkpoint blockade. *Nat Commun* (2020) 11(1):4835. doi: 10.1038/s41467-020-18570-x

83. Lucas ED, Schafer JB, Matsuda J, Kraus M, Burchill MA, Tamburini BAJ. PD-L1 reverse signaling in dermal dendritic cells promotes dendritic cell migration required for skin immunity. *Cell Rep* (2020) 33(2):108258. doi: 10.1016/j.celrep.2020.108258

84. Gordon SR, Maute RL, Dulken BW, Hutter G, George BM, McCracken MN, et al. PD-1 expression by tumour-associated macrophages inhibits phagocytosis and tumour immunity. *Nature.* (2017) 545(7655):495–9. doi: 10.1038/nature22396

85. Hartley GP, Chow L, Ammons DT, Wheat WH, Dow SW. Programmed cell death ligand 1 (PD-L1) signaling regulates macrophage proliferation and activation. *Cancer Immunol Res* (2018) 6(10):1260–73. doi: 10.1158/2326-6066.CIR-17-0537

86. Yoshida K, Okamoto M, Sasaki J, Kuroda C, Ishida H, Ueda K, et al. Anti-PD-1 antibody decreases tumour-infiltrating regulatory T cells. *BMC Cancer.* (2020) 20(1):25. doi: 10.1186/s12885-019-6499-y

87. Zappasodi R, Serganova I, Cohen IJ, Maeda M, Shindo M, Senbabaoglu Y, et al. CTLA-4 blockade drives loss of t(reg) stability in glycolysis-low tumours. *Nature.* (2021) 591(7851):652–8. doi: 10.1038/s41586-021-03326-4

88. Selby MJ, Engelhardt JJ, Quigley M, Henning KA, Chen T, Srinivasan M, et al. Anti-CTLA-4 antibodies of IgG2a isotype enhance antitumor activity through reduction of intratumoral regulatory T cells. *Cancer Immunol Res* (2013) 1(1):32–42. doi: 10.1158/2326-6066.CIR-13-0013

89. Sun C, Mezzadra R, Schumacher TN. Regulation and function of the PD-L1 checkpoint. *Immunity.* (2018) 48(3):434–52. doi: 10.1016/j.immuni.2018.03.014

90. Zhao Y, Chen S, Lan P, Wu C, Dou Y, Xiao X, et al. Macrophage subpopulations and their impact on chronic allograft rejection versus graft acceptance in a mouse heart transplant model. *Am J Transplant.* (2018) 18(3):604–16. doi: 10.1111/ajt.14543

91. Ono Y, Perez-Gutierrez A, Nakao T, Dai H, Camirand G, Yoshida O, et al. Graft-infiltrating PD-L1hicross-dressed dendritic cells regulate antidonor T cell responses in mouse liver transplant tolerance. *Hepatology.* (2018) 67(4):1499–515. doi: 10.1002/hep.29529

92. Bracamonte-Baran W, Gilotra NA, Won T, Rodriguez KM, Talor MV, Oh BC, et al. Endothelial stromal PD-L1 (Programmed death ligand 1) modulates CD8(+) T-cell infiltration after heart transplantation. *Circ Heart Fail* (2021) 14(10):e007982. doi: 10.1161/CIRCHEARTFAILURE.120.007982

93. Matsushima H, Morita-Nakagawa M, Datta S, Pavicic PGJr., Hamilton TA, Abu-Elmagd K, et al. Blockade or deficiency of PD-L1 expression in intestinal allograft accelerates graft tissue injury in mice. *Am J Transplant.* (2022) 22(3):955–65. doi: 10.1111/ajt.16873

94. Dixon KO, Tabaka M, Schramm MA, Xiao S, Tang R, Dionne D. Tim3 blockade in dendritic cells may potentiate antitumor immunity. *Cancer Discovery* (2021) 11(8):1873. doi: 10.1158/2159-8290.CD-RW2021-084

95. Lichtenegger FS, Rothe M, Schnorfeil FM, Deiser K, Krupka C, Augsberger C, et al. Targeting LAG-3 and PD-1 to enhance T cell activation by antigen-presenting cells. *Front Immunol* (2018) 9:12. doi: 10.3389/fimmu.2018.00385

96. Wang Y, Zhou S, Yang F, Qi X, Wang X, Guan X, et al. Treatment-related adverse events of PD-1 and PD-L1 inhibitors in clinical trials: A systematic review and meta-analysis. *JAMA Oncol* (2019) 5(7):1008–19. doi: 10.1001/jamaoncol.2019.0393

97. Bai X, Wang X, Ma G, Song J, Liu X, Wu X, et al. Improvement of PD-1 blockade efficacy and elimination of immune-related gastrointestinal adverse effect by mTOR inhibitor. *Front Immunol* (2021) 12:793831. doi: 10.3389/fimmu.2021.793831

98. Esfahani K, Al-Aubodah T-A, Thebault P, Lapointe R, Hudson M, Johnson NA, et al. Targeting the mTOR pathway uncouples the efficacy and toxicity of PD-1 blockade in renal transplantation. *Nat Commun* (2019) 10(1):4712. doi: 10.1038/s41467-019-12628-1

99. Lipson EJ, Bagnasco SM, Moore J, Jang S, Patel MJ, Zachary AA, et al. Tumor regression and allograft rejection after administration of anti-PD-1. *New Engl J Med* (2016) 374(9):896–8. doi: 10.1056/NEJMc1509268

frontiers | Frontiers in Pharmacology

TYPE Review
PUBLISHED 21 March 2023
DOI 10.3389/fphar.2023.1138493



OPEN ACCESS

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SPECIALTY SECTION
This article was submitted to Gastrointestinal and Hepatic Pharmacology, a section of the journal Frontiers in Pharmacology

RECEIVED 05 January 2023
ACCEPTED 09 March 2023
PUBLISHED 21 March 2023

CITATION
Sun L-Y, Zhang K-J, Xie Y-M, Liu J-W and Xiao Z-Q (2023), Immunotherapies for advanced hepatocellular carcinoma. *Front. Pharmacol.* 14:1138493. doi: 10.3389/fphar.2023.1138493



# Immunotherapies for advanced hepatocellular carcinoma

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Primary liver cancer is the second leading cause of tumor-related deaths in China, with hepatocellular carcinoma (HCC) accounting for 80%–90% of these. Since there is a lack of symptoms in the early stages of HCC, a large proportion of patients were identified with unresectable HCC when diagnosed. Due to the severe resistance to chemotherapy, patients with advanced HCC were traditionally treated with systematic therapy in the past decades, and the tyrosine kinase inhibitor (TKI) sorafenib has remained the only treatment option for advanced HCC since 2008. Immunotherapies, particularly immune checkpoint inhibitors (ICIs), have shown a strong anti-tumor effect and have been supported by several guidelines recently. ICIs, for example programmed cell death-1 (PD-1) inhibitors such as nivolumab and pembrolizumab, programmed cell death ligand 1 (PD-L1) inhibitors such as atezolizumab, and cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) inhibitors such as ipilimumab, the ICI-based combination with TKIs, and VEGF-neutralizing antibody or systematic or local anti-tumor therapies, are being further studied in clinical trials. However, immune-related adverse events (irAEs) including cutaneous toxicity, gastrointestinal toxicity, and hepatotoxicity may lead to the termination of ICI treatment or even threaten patients' lives. This review aims to summarize currently available immunotherapies and introduce the irAEs and their managements in order to provide references for clinical application and further research.

KEYWORDS

**hepatocellular carcinoma, immunotherapy, PD-1, PD-L1, CTLA-4**

## Introduction

Liver cancer is a concerning health challenge and is the sixth most common malignancy and the fourth leading cause of cancer-related mortality worldwide (Villanueva, 2019; Llovet et al., 2021a). HCC, which is generally attributed to the background of chronic liver diseases including hepatitis B virus (HBV) or hepatitis C virus (HCV) infection and alcoholic liver disease or non-alcoholic fatty liver disease (NAFLD), accounts for over 90% of liver cancers

**Abbreviation:** HCC, hepatocellular carcinoma; HBV, hepatitis B virus; HCV, hepatitis C virus; NAFLD, non-alcoholic fatty liver disease; OS, overall survival; TKI, tyrosine kinase inhibitor; PD-1, programmed cell death-1; PD-L1, programmed cell death ligand 1; CTLA-4, cytotoxic T-lymphocyte-associated protein 4; ICI, immune checkpoint inhibitor; NSCLC, non-small-cell lung cancer; FDA, Food and Drug Administration; RECIST, Response Evaluation Criteria in Solid Tumors; ORR, objective response rate; irAEs, immune-related adverse events; PFS, progression-free survival; BSC, best supportive care; TTP, time-to-time progression.

(Younossi et al., 2018; Llovet et al., 2021b). Although the incidence rates of HCC have decreased due to the coverage of HBV vaccines and anti-viral therapies in some regions, the global incidence of HCC continues to rise, resulting in at least 1,000,000 HCC cases annually by 2025 (Llovet et al., 2021a; Sung et al., 2021). Unfortunately, due to the lack of symptoms and physical characteristics of HCC patients, as well as the unsatisfactory HCC surveillance accuracy and popularity, potentially curative treatment is not possible for over 80% of patients at the time of diagnosis (Zongyi and Xiaowu, 2020). Due to the severe and broad resistance to cytotoxic chemotherapy, systemic therapy was a controversial option for patients with advanced HCC before 2008. After years of waiting and many unsuccessful clinical trials, Llovet et al. (2008) demonstrated the anti-tumor effect of sorafenib as an oral multi-kinase inhibitor in a phase III trial, the SHARP study. Sorafenib was the first systemic therapy for HCC, prolonging survival by a few months. Although the survival benefit of using sorafenib is not clinically meaningful, the viable option for advanced HCC was limited to sorafenib alone for 10 years until the emergence of lenvatinib, which not only showed an overall survival (OS) that was not inferior to sorafenib but also improved all secondary endpoints (Al-Salama et al., 2019). Moreover, regorafenib was also approved as the second-line therapeutic setting for advanced HCC (Llovet et al., 2018).

In addition to TKIs including sorafenib, lenvatinib, and regorafenib, immunotherapy is gaining continued traction in treating advanced HCC (Fulgenzi et al., 2021). Based on the cancer immunosurveillance hypothesis postulating that evasion from immune control is an essential feature of cancer, immune checkpoint molecules including PD-1, PD-L1, and CTLA-4 were further studied (Brahmer et al., 2010; 2012; Pardoll, 2012; Topalian et al., 2012; Zitvogel et al., 2016a; Zitvogel et al., 2016b). In fact, ICIs have been proven to be an efficacious anti-cancer strategy in other solid cancers, e.g., non-small-cell lung cancer (NSCLC), renal cancer, and melanoma (Larkin et al., 2015; Amin and Hammers, 2018; Mazieres et al., 2019). Recent clinical trials have also discovered the prolonged survival of HCC patients using ICIs, showing the promising curative effect of ICIs toward HCC (Finn et al., 2020a; Greten et al., 2021; Yau et al., 2022). As a breakthrough, the combination of atezolizumab plus bevacizumab was introduced into the first-line therapies for advanced HCC, which has provided patients with a hopeful option (Llovet et al., 2021b).

## Mechanisms of immunotherapy

In the normal cancer-immunity cycle for killing tumor cells (Figure 1), the antigens from tumor cells are first captured and further processed by dendritic cells (DCs). Second, captured antigens are presented to T cells to activate the T-cell responses against the cancer-specific antigens (Chen and Mellman, 2013). After assembling in the tumor tissue and infiltrating the tumor bed, T cells specifically recognize and bind tumor cells and then kill the targeted tumor cells (Chen and Mellman, 2013). However, in tumor patients, the cancer-immunity cycles fail to run optimally, leading to tumor development and even endangering the host's life.

Belonging to the immunoglobulin super family, PD-1 is a transmembrane coinhibitory receptor primarily expressed on the surface of activated T cells and NK cells (Huang et al., 2021) as the ligands to PD-1, PD-L1 (B7-H1 or CD274), and PD-L2 (B7-DC or CD273) are expressed on the surface of tumor cells (Figure 2) (Huang et al., 2021). Once the tumor cells are detected by the

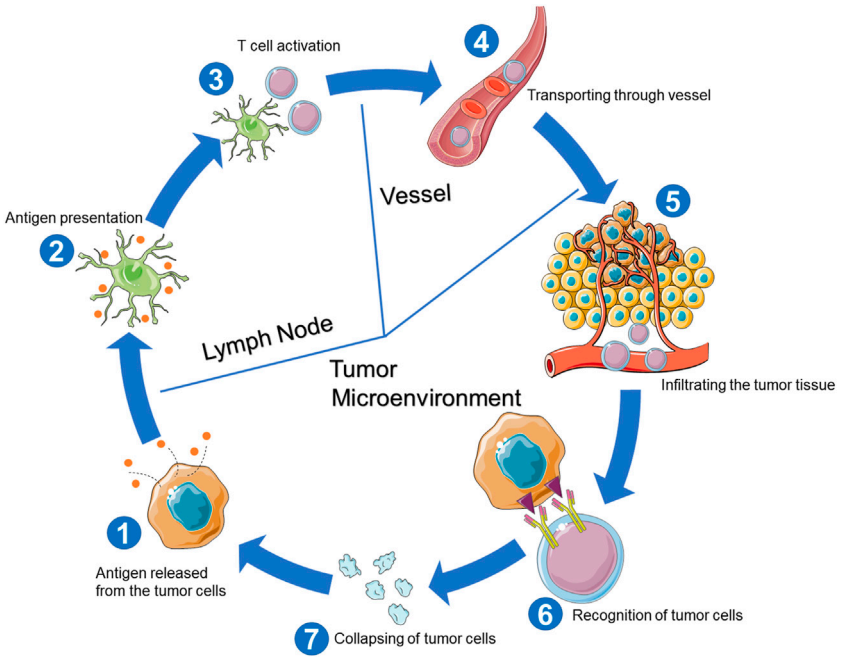


FIGURE 1
Cancer-immunity cycle for killing tumor cells.

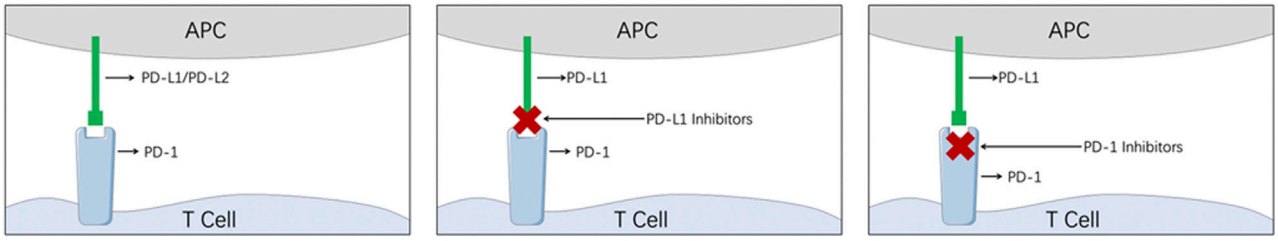


FIGURE 2
Illustration of the mechanism of PD-1/PD-L1 inhibitors. (APC, antigen-presenting cell; PD-1, programmed cell death-1; PD-L1, programmed cell death ligand 1).

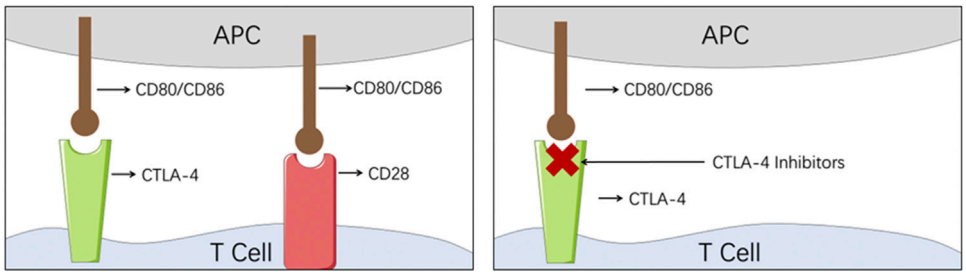


FIGURE 3
Illustration of the mechanism of CTLA-4 inhibitors. (APC, antigen-presenting cell; CTLA-4, cytotoxic T-lymphocyte-associated protein 4).

T cells, the overexpressed PD-L1/2 from the tumor cells engages with PD-1 on the T cells, and the physiological inhibitory pathways will therefore be hijacked by the tumor cells to escape the host immune surveillance system (Huang et al., 2021). After numerous attempts, the PD-1/PD-L1 inhibitors were proved to have the ability to remove the coinhibitory signal by blocking PD-1 or PD-L1, and rebuild the normal immune system surveillance kill tumor cells (Sharma and Allison, 2015).

CTLA-4, as a member of the CD28 immunoglobulin subfamily, is also mainly expressed on the T cells (Figure 3). When CTLA-4 engages with its ligands, CD80 and CD86, similarly to CD28, the coinhibitory response will be activated and the tumor cell will escape the host immune surveillance system (Rowshanravan et al., 2018; Xu et al., 2018). On the contrary, when CD80 and CD86 engage with CD28, the costimulatory response is initiated (Rowshanravan et al., 2018; Xu et al., 2018). Therefore, by blocking the checkpoint CTL4-4, CTLA-4 inhibitors managed to repair the collapsed immune surveillance system.

## PD-1/PD-L1 inhibitors

The engagement of PD-1 expressed on the surface of activated $CD8^+$ T cells with PD-L1 expressed by HCC cells not only averts the excessive activation of T cells, decreasing tumor-killing efficiency by transmitting inhibitory signals, but also weakens proliferation and cytolytic activity, followed by the defects or even deletion of cytokine production, eventually leading to an exhausted T-cell phenotype (Wherry, 2011). With regard to the mechanisms of the PD-1 engagement with PD-L1 in the development of HCC, PD-1 and PD-L1 inhibitors are widely recognized as the backbone of systemic therapies for HCC, and several main randomized clinical trials are shown in Table 1.

In 2007, according to the results of the CheckMate 040 trial, nivolumab was granted accelerated approval by the US Food and Drug Administration (FDA) as a PD-1 inhibitor for treating advanced HCC after the failure of sorafenib (Chiew Woon et al., 2020). In the CheckMate 040 trial, 214 patients in the dose-expansion phase and 48 patients in the dose-escalation phase were enrolled (El-Khoueiry et al., 2017). According to the Response Evaluation Criteria in Solid Tumors (RECIST) 1.1 criteria, an objective response rate (ORR) of 20% (95% CI: 15%–26%) was shown in the dose-expansion phase at the nivolumab dose of 3 mg/kg every 2 weeks and an ORR of 15% (95% CI: 6%–28%) was shown in the dose-escalation phase (El-Khoueiry et al., 2017). Among 48 patients in the dose-escalation phase, the median duration of response to nivolumab was 17 months (95% CI: 6–24 months), and among responders, a 2-year survival rate of over 80% was observed (El-Khoueiry et al., 2017).

The efficacy of nivolumab was further evaluated in the CheckMate 459 trial by comparing it with sorafenib, which was the first systemic agent approved for the treatment of HCC (Man et al., 2021; Yau et al., 2022). In this randomized, open-label, phase III clinical trial, 743 patients across 22 countries and regions were finally selected and randomly assigned into two cohorts (nivolumab, n = 371; sorafenib, n = 372) (Yau et al., 2022). At the follow-up after

**TABLE 1 Main randomized clinical trials of ICIs for advanced HCC.**

| Name | Treatment | Study phase | Control group | Primary endpoint | ORR, % | Median OS, months | Median PFS, months |
|---|---|---|---|---|---|---|---|
| CheckMate 040 | Nivolumab | I/II | None | Safety and ORR | 20 | 15.6 | 4.0 |
| CheckMate 040 | Nivolumab plus ipilimumab | I/II | None | Safety, tolerability, and ORR | 32 | 22.8 | NR |
| KEYNOTE-224 | Pembrolizumab | II | None | ORR | 17 | 12.9 | 4.9 |
| KEYNOTE-240 | Pembrolizumab | III | Placebo | OS and PFS | 18.3 vs. 14.4, $p < 0.001$ | 13.8 vs. 10.6, $p = 0.024$ | 3.0 vs. 2.8, $p = 0.002$ |
| CheckMate 459 | Nivolumab | III | Sorafenib | OS | 15 vs. 7, $p$ = NR | 16.4 vs. 14.8, $p = 0.052$ | 3.7 vs. 3.8, $p$ = NS |
| IMbrave150 | Atezolizumab plus bevacizumab | III | Sorafenib | OS and PFS | 30 vs. 11, $p < 0.001$ | 19.2 vs. 13.4, $p < 0.001$ | 6.8 vs. 4.3, $p < 0.001$ |
| COSMIC-312 | Cabozantinib plus atezolizumab | III | Sorafenib | OS and PFS | 13 vs. 6, $p$ = NR | 15.4 vs. 15.5, $p = 0.44$ | 6.8 vs. 4.2, $p = 0.001$ |

ICI, immune checkpoint inhibitor; NR, not reported; NS, not significant; ORR, objective response rate; OS, overall survival; PFS, progression-free survival.

22.8 months, the nivolumab cohort achieved a median OS of 16.4 months (95% CI: 13.9–18.4 months) *versus* the sorafenib cohort that achieved a median OS of 14.7 months (95% CI: 11.9–17.2 months). Despite an extra 2 months of OS time, with a *p*-value of 0.075, the CheckMate 459 trial did not meet the primary boundary. However, given the fact that at least 31% of patients from the sorafenib cohort had received ICIs after sorafenib treatment, as well as the secondary endpoints favoring nivolumab over sorafenib, the study still concluded that nivolumab was superior to sorafenib, with encouraging long-term survival, durable clinical activity of response frequency and durability, less immune-related adverse events, and clinically meaningful improvements in health-related quality of life (Sangro et al., 2021; Yau et al., 2022).

Similar to nivolumab, pembrolizumab is another fully humanized PD-1 monoclonal antibody inhibitor. A year after the approval of nivolumab, considering the results from a non-randomized, multicenter, open-label, phase II trial "KEYNOTE-224" reported in 2018, the FDA approved pembrolizumab for the treatment of advanced HCC after sorafenib failure or intolerance (Zhu et al., 2018). After recruitment and screening, 104 patients with advanced HCC after sorafenib treatment were finally enrolled into this study. The primary endpoint was an objective response, and it was shown that the objective response was observed in 18 patients (17%), among which the best overall responses were complete response from one patient (1%) and partial responses from 17 patients (16%) (Zhu et al., 2018). Meanwhile, the median OS was 12.9 months (95% CI: 11.9–17.2 months), the median progression-free survival (PFS) was 4.9 months (95% CI: 3.9–8.0 months), the 1-year PFS rate was 28% (95% CI: 19%–37%), and the 1-year OS rate was 54% (95% CI: 44%–63%) (Zhu et al., 2018).

However, the trial "KEYNOTE-224" was a non-randomized study without a control group, and the results were further validated in a large randomized, phase III trial "KEYNOTE-240" (Finn et al., 2020b). In 2020, the results of KEYNOTE-240 were reported. The efficacy of pembrolizumab was further evaluated by comparing with the control cohort using best supportive care (BSC) or placebo plus BSC, and the primary endpoint was OS and PFS. A total of 413 advanced HCC patients from 119 institutions across 27 countries were finally recruited and divided into the pembrolizumab cohort (n = 278) and the placebo cohort (n = 135) (Finn et al., 2020c). It was reported that the median OS of the pembrolizumab cohort was 13.9 months (95% CI: 11.6–16.0 months), which was better than the median OS of 10.6 months (95% CI: 8.3–13.5 months) from the placebo cohort with a *p*-value of 0.0238. In terms of tumor progression, the pembrolizumab cohort showed a median PFS of 3.0 months (95% CI: 2.8–4.1 months), which was superior to that of 2.8 months (95% CI: 1.6–3.0 months) from the placebo cohort ($p$ = 0. 0022) (Finn et al., 2020a). Although both the OS and PFS were improved after pembrolizumab treatment compared to the placebo cohort, the trial "KEYNOTE-240" was still judged as a failure as it did not meet the prespecified statistical endpoints.

As shown previously, the ORR of several PD-1 inhibitors was only 15%–20%, and the first-line monotherapy trial "CheckMate 459" and the second-line monotherapy trial "KEYNOTE-240" were both declared failures (Finn et al., 2020b; Yau et al., 2022). It was not until the emergence of IMbrave150 that hope was revived for the systematic treatment of HCC. With the publication of this global, open-label, phase III randomized trial, the combination of the PD-L1 inhibitor, atezolizumab, and the anti-VEGF monoclonal antibody, bevacizumab, was highly expected as a novel strategy for unresectable HCC treatment (Finn et al., 2020c).

Before IMbrave 250, atezolizumab treatment had been validated as a superior option to platinum-based chemotherapy for NSCLC patients with high PD-L1 expression (Herbst et al., 2020). In this trial, a total of 501 patients with locally advanced metastatic or unresectable HCC were finally enrolled, of which 336 (67.1%) patients were randomly assigned to receive atezolizumab plus bevacizumab, while 165 (32.9%) patients were included in the sorafenib cohort. Defining OS and PFS as the primary endpoints, patients of the atezolizumab–bevacizumab cohort conducted better

estimated survival rates at timepoints of 6 months (84.8% *versus.* 72.2%) and 12 months (67.2% *versus.* 54.6%) compared to the sorafenib cohort (Finn et al., 2020a). Meanwhile, the atezolizumab–bevacizumab cohort also had a significantly longer PFS than the sorafenib cohort (median, 6.8 months *versus.* 4.3 months, $p < 0.001$). Furthermore, the PFS at 6 months in the atezolizumab–bevacizumab cohort was 54.5%, which was much higher than 37.2% in the sorafenib cohort. Not only the primary endpoints but also the secondary endpoints of the atezolizumab–bevacizumab cohort performed better than the sorafenib cohort. The confirmed ORR was 27.5% (95% CI: 22.5%–32.5%) in the atezolizumab–bevacizumab cohort, which was significantly superior to that of 11.9% (95% CI: 7.4%–18.0%) in patients treated with sorafenib ($p < 0.001$).

## CTLA-4 inhibitors

Similar to PD-1, CTLA-4 is another member of the immunoglobulin-related receptor family regulating various aspects of T-cell immune functions (Zhang et al., 2019). CTLA-4 is mainly expressed in regulatory T cells, which transmits a negative signal directly in effector T cells and regulates the negative immune responses of T cells (Lisi et al., 2022). Therefore, CTLA-4 has been envisioned as a target of monoclonal antibodies for cancer immunotherapy and CTLA-4 inhibitors. To enhance its anti-tumor effect, nowadays, the CTLA-4 inhibitors are widely used in combination with other ICIs (Yau et al., 2020; Pinato et al., 2021a).

Ipilimumab was the first CTLA-4 inhibitor approved in 2010 for metastatic melanoma (Hodi et al., 2010). Meanwhile, tremelimumab was the first CTLA-4 inhibitor used for HCC treatment (Sangro et al., 2013). In the clinical trial conducted by Sangro et al., 20 HCV-positive patients with inoperable HCC were enrolled and received intravenous tremelimumab at a dose of 15 mg/kg on day 1 of every 90-day cycle until tumor progression and occurrence of unacceptable toxicities. The trial showed that under tremelimumab treatment, patients with inoperable HCC achieved a median OS of 8.2 months and a median time-to-progression (TTP) of 6.48 months. Moreover, the 6-month survival rate was 64% and the 1-year survival rate was 43%.

## Exploration of ICI combinations

Apart from the combination of atezolizumab and bevacizumab, several studies investigated the possibility of combining ICIs of different targets. As it was mentioned previously, CTLA-4 ICIs were usually used in combination with PD-1/PD-L1 ICIs. In the CheckMate 040 trial, a total of 148 patients were enrolled to receive the combination of ipilimumab and nivolumab (Yau et al., 2020). In this multicenter, open-label, phase I/II study, patients were randomly divided into three arms (50 in arm A and 49 each in arms B and C). The dose of ipilimumab–nivolumab differed across different arms. Patients in arm A were treated with nivolumab 1 mg/kg plus ipilimumab 3 mg/kg every 3 weeks, followed by nivolumab 240 mg every 2 weeks; patients in arm B were treated with nivolumab 3 mg/kg plus ipilimumab 1 mg/kg every 3 weeks, followed by nivolumab 240 mg every 2 weeks; and patients in arm C were treated with nivolumab 3 mg/kg every 2 weeks plus ipilimumab 1 mg/kg every 6 weeks. After follow-ups, arm A showed the highest ORR of 32% (95% CI: 20%–47%) compared with 27% (95% CI: 15%–41%) in arm B and 29% (95% CI: 17%–43%) in arm C and the longest median OS of 22.8 months *versus* 12.5 months and 12.7 months in arms B and C, respectively.

In addition to the combination of different ICIs, the combination of ICIs and TKIs is also a potentially effective treatment for advanced HCC. TKIs play an anti-tumor role by blocking several angiogenic pathways and further maintaining the consequent stability of the vascular endothelium in the tumor bed (Wong et al., 2015). TKIs, along with ICIs, have been considered the cornerstone for systematic HCC treatment. Since 2007, several TKIs, including sorafenib and lenvatinib, have been approved for the systemic treatment of advanced HCC (Al-Salama et al., 2019). Research on exploring the efficacy of the combination of TKIs and ICIs in the treatment of advanced HCC has never stopped. An international, open-label, randomized clinical phase III trial named COSMIC-312, which studied the combination of cabozantinib and atezolizumab, was recently published (Antonella et al., 2022; Kelley et al., 2022). A total of 837 advanced HCC patients have been enrolled and randomly treated with cabozantinib–atezolizumab, sorafenib alone, or cabozantinib alone in a 2:1:1 ratio. Researchers assessed the PFS in accordance with RECIST 1.1 that was assessed by a blinded and independent committee for the first 372 patients from the cabozantinib–atezolizumab cohort or sorafenib cohort and OS in all the patients from the cabozantinib–atezolizumab cohort or sorafenib cohort as the dual primary endpoints of this study. It was reported that the combination treatment cohort achieved a median PFS of 6.8 months (95% CI: 5.6–8.3 months) *versus* 4.2 months (95% CI: 2.8–7.0 months) in the sorafenib cohort with a statistically significant *p*-value of 0.0012. However, in the interim analysis, the median OS in the cabozantinib–atezolizumab cohort was 15.4 months (95% CI: 13.7–17.7 months), while the median OS in the sorafenib cohort was 15.5 months (12.1- not estimable) with a *p*-value of 0.44. Additionally, in subgroups with more advanced HCC, an improved PFS was also observed, and further studies to evaluate the efficacy of cabozantinib plus atezolizumab are still needed.

## Ongoing clinical trials

Needless to say, the research focusing on the immune checkpoint inhibitors is far from over, and there are abundant clinical trials ongoing, exploring efficient immunotherapies. An abstract of the clinical trial "RATIONALE 301" exploring the efficiency of tislelizumab *versus* sorafenib for advanced HCC was reported recently. The study revealed that patients receiving tislelizumab showed an OS not inferior to that of those receiving sorafenib (15.9 months *versus.* 14.1 months), and the tislelizumab cohort showed a better ORR (14.3% *versus.* 5.4%). Meanwhile, fewer patients in the tislelizumab cohort experienced irAEs, and fewer patients suffered irAEs that led to discontinuation or dosing adjustment. Another clinical trial (NCT03764293) evaluated the

efficiency and safety of the combination of camrelizumab with rivoceranib for unresectable HCC compared with those of sorafenib. The combination cohort showed both significantly longer median OS (22.1 months *versus.* 15.2 months) and median PFS (5.6 months *versus.* 3.7 months). Notably, the combination of camrelizumab with rivoceranib achieved the longest median OS among all the phase III clinical trials for advanced HCC, and this combination has the potential to be another first-line treatment option.

## Adverse events after ICI treatments

Although the therapeutics for advanced HCC were reshaped, the immune-modulatory therapy inevitably leads to immune system imbalance and a series of irAEs including cutaneous toxicity, gastrointestinal toxicity, hepatotoxicity, and thyroiditis (Mitchell et al., 2013; Khoja et al., 2017; Wu et al., 2017; Barroso-Sousa et al., 2018; Kurimoto et al., 2020; Pinato et al., 2021b). Unfortunately, the precise mechanism of irAEs still remains unclear. These irAEs tend to appear after 8 weeks of ICI treatment, and most of them are typically reversible and controllable, but occasionally they lead to withdrawal or fatal outcomes. Therefore, monitoring and managing such irAEs are also an essential part of ICI therapeutic strategies, and most clinical trials considered the occurrence of irAEs as one of the endpoints of the whole trials.

In terms of irAEs in all the cancers, irAEs after PD-1 or PD-L1 inhibitor treatment are dose-independent, while in those treated with the CTLA-4 inhibitor, the occurrence of irAEs tends to be dose-dependent (Bertrand et al., 2015; Wang et al., 2019). Two independent meta-analyses on PD-L1 and CTLA-4 reached similar conclusions that the most common target organs for irAEs are skin, followed by the gastrointestinal tract and liver (Bertrand et al., 2015; Wang et al., 2018). Meanwhile, for patients treated with PD-1/PD-L1 inhibitors in combination with chemotherapy, radiotherapy, immunotherapy, or targeted therapy, a meta-analysis identified that the most common irAEs of all grades were anemia (45.4%), fatigue (combination with targeted therapy) (34.3%), fatigue (combination with targeted therapy) (26.4%), and dysphagia ((30.0%), respectively, and the most common irAEs of grade 3 or higher were neutropenia (19.6%), hypertension (9.3%), a high level of lipase (7.2%), and lymphopenia (10.3%) (Zhou et al., 2021). However, due to the unique liver immunobiology and underlying liver diseases such as cirrhosis and viral hepatitis in HCC patients, the symptoms of irAEs were always covered or ignored, which poses a major challenge to the safe use of ICIs for advanced HCC patients.

Cutaneous toxicity is the most common and obvious irAEs after ICI treatment. Generally, cutaneous toxicity mostly manifesting as rash and pruritus occurs within 2 weeks after the first dose. Fortunately, less than 1% of patients receiving monotherapies and 4% of patients receiving combination therapies develop skin irAEs of grade 3 or higher (Sangro et al., 2020). According to previous studies, rash occurred in 15%–30% of patients receiving nivolumab alone, 8%–10% of patients receiving pembrolizumab alone, and 17%–29% of patients receiving a combination of nivolumab and ipilimumab. Meanwhile, pruritus occurred in 20%–27% of patients in the nivolumab cohort, 12%–18% of patients in the pembrolizumab cohort, and 30%–45% of those treated with the combination. For patients with dermatological problems after receiving ICIs, first of all, pre-existing skin conditions, chronic liver disease-related skin disorder, or any other causes of skin disorder should be identified and ruled out. For patients with cutaneous involvement of grade 1 or 2, ICI treatment can be continued after administering triamcinolone 0.1% along with antihistamine treatment. For patients with more severe symptoms (grade 3), systemic hormone therapies, such as oral prednisolone, should be given at a dose of 1–2 mg/kg on the basis of the aforementioned topical therapy. For patients with grade 4 or life-threatening skin disorders, ICI treatment should be terminated immediately and methylprednisolone should be given at a dose of 1–2 mg/kg (Brahmer et al., 2018).

Gastrointestinal toxicity in patients after ICI treatments usually manifests as diarrhea and colitis (Vogl et al., 2011; Nielsen et al., 2022). Generally, diarrhea and colitis are commonly diagnosed at 6–8 weeks, following the initiation of ICIs. For overall cancer populations, a recently published meta-analysis showed that the incidence rates of diarrhea of grade 1–4 and grade 3–4 after administering pembrolizumab at a dose of 200 mg every 3 weeks were 9.5% and 0.3%, and the incidence rates of colitis of grade 1–4 and grade 3–4 were 1.3% and 0.4%, respectively (Nielsen et al., 2022). Meanwhile, at the standard flat dose of nivolumab of 240 mg every 2 weeks, the incidence rates of diarrhea of grade 1–4 and grade 3–4 were 11.6% and 0.04%, and the incidence rates of colitis of grade 1–4 and grade 3–4 were 0.2% and 0.0%, respectively (Nielsen et al., 2022). For patients receiving a 1,200 mg dosage of atezolizumab every 3 weeks, the incidence rate of grade 1–4 and grade 3–4 diarrhea was 8.8% and 0.1%, and 0.6% and 0.3% for grade 1–4 and grade 3–4 colitis, respectively. For advanced HCC patients after ICI treatments, the incidence of diarrhea and colitis is consistent with that of the overall tumor populations. Similar to managing cutaneous toxicity, the first step in dealing with ICI-related gastrointestinal toxicity is identifying the cause of diarrhea and colitis including underlying diseases or medications that induce diarrhea, such as lactulose. Generally, colonoscopy still remains the gold diagnostic standard of gastrointestinal toxicity and contributes to severity assessment grading. As for the treatments, once gastrointestinal toxicity is identified, severity grading should be assessed by symptoms or colonoscopy first. For diarrhea of grade 1, no special treatment is needed except strengthening monitoring, and ICI treatment can be continued. Symptomatic treatments, such as parenteral administration of fluids and electrolytes, are warranted. Oral corticosteroids at a dose of 0.5–1 mg/kg should be given if diarrhea or colitis of grade 2 persisted for over 3 days, and intravenous corticosteroids are needed for gastrointestinal toxicity of grade 3 or higher. Meanwhile, ICI treatments should be terminated when patients are diagnosed with diarrhea or colitis of grade 2–3, and ICI treatment should be terminated permanently when gastrointestinal toxicity of grade 4 is identified.

Since patients with advanced HCC are usually diagnosed with underlying chronic liver diseases or liver dysfunction, hepatotoxicity, which always manifests as hepatitis or elevations of liver enzymes after ICI treatments, is a relatively frequent irAE. Compared with other types of tumors, including melanoma and NSCLC, a higher proportion of liver enzyme increase occurred after ICI treatment in HCC (Vogl et al., 2011; Brown et al., 2017; Lleo

et al., 2019; De Martin et al., 2020). Elevations of liver enzymes were found in 13% of patients receiving pembrolizumab in the trial "KEYNOTE-224" and 16% of patients receiving nivolumab plus ipilimumab in the trial "CheckMate 040" (Zhu et al., 2018; Yau et al., 2020). Patients after ICI treatments should undergo regular liver function examinations as the hepatitis or liver dysfunction tend to be asymptomatic and progress rapidly to liver failure at later stages. Hepatotoxicity commonly occurs at 4–12 weeks after the initial ICI treatment. For patients with ICI-related hepatotoxicity, steroid therapy is not necessarily required, and ICIs can be continued or delayed if patients were identified with asymptomatic liver enzyme elevation or irAEs of grade 1–2. As for patients with hepatotoxicity of grade ≥3, the level of liver enzymes mostly returns to normal after timely steroid therapy, and ICI can be reintroduced when the level of aminotransferases declines or returns to baseline levels (De Martin et al., 2018).

Thyroiditis related to ICI treatment is generally assumed as the main etiology of thyroid dysfunction, which is the most commonly observed endocrine gland irAE (Muir et al., 2021). The symptoms of thyroid dysfunction vary, including hyperthyroidism and hypothyroidism, of which hypothyroidism accounts for the majority. The diagnosis of thyroid dysfunction is mainly based on the comparison of thyroid hormone levels before and after ICI treatment (Illouz et al., 2018). The incidence of thyroid dysfunction varies due to the different types of ICIs. It was reported in a phase III clinical trial that the incidence of hypothyroidism was 13.0% in advanced HCC patients after receiving a PD-1 inhibitor and 22.2% after receiving a combination of PD-1 inhibitor and CTLA-4 (ipilimumab) (Morganstein et al., 2017). Meanwhile, the general thyroid dysfunction rate was 29% after ipilimumab treatment, 18% after PD-1 treatment, and 50% after receiving the combination (Morganstein et al., 2017). It is worth mentioning that a considerable number of patients who develop hypothyroidism have a temporary symptom of hyperthyroid at the initial phase, which highlights the importance of timely recognition and careful nursing to avoid medical negligence. Unfortunately, the pathogenesis of developing ICI-related thyroiditis still remains unknown. According to a previous study conducted by Muir et al. (2021), female individuals, younger patients, and those who undergo combination of PD-1 and CTLA-4 inhibitors have higher possibilities of developing thyroiditis. For HCC patients undergoing ICI treatments, screening for thyroid-stimulating hormone and thyroxine regularly is necessary, which allows doctors to diagnose thyroid dysfunction when the patient is still asymptomatic. In general, hypothyroidism related to ICI does not lead to the termination of treatment, and an incremental thyroid replacement therapy at a dose of 25–50 μg is adequate for treating symptomatic hypothyroidism (Sangro et al., 2013). For patients developing hyperthyroidism after ICI treatment, consultation with an endocrinologist is recommended and the heart rate should be maintained below 90 bpm (Sangro et al., 2013).

## Summary

The establishment of immunotherapy has reshaped the treatment paradigm for advanced HCC in the past decades, and more immune checkpoints, as well as the combination therapies, are being studied further. Although related to a wide range of irAEs, immunotherapy remains the key point of future research, with the hope of overcoming cancer.

## Author contributions

L-YS: ideas, formulation or evolution of overarching research goals and aims, preparation, and writing the initial draft; K-JZ: critical review and commentary or revision; Y-MX: ideas, creation and/or presentation of the published work, and writing the initial draft; J-WL and Z-QX: critical review, commentary or revision, and mentorship external to the core team.

## Funding

This work was supported by the funding from the "2021 Zhejiang Traditional Chinese Medicine Scientific Research Foundation Project (Class A): 2021ZA125" for J-WL.

## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

## Publisher's note



## References

Al-Salama, Z. T., Syed, Y. Y., and Scott, L. J. (2019). Lenvatinib: A review in hepatocellular carcinoma. *Drugs* 79 (6), 665–674. doi:10.1007/s40265-019-01116-x

Amin, A., and Hammers, H. (2018). The evolving landscape of immunotherapy-based combinations for frontline treatment of advanced renal cell carcinoma. *Front. Immunol.* 9, 3120. doi:10.3389/fimmu.2018.03120

Antonella, C., Valentina, Z., Antonio, D., Tiziana, P., Nicola, P., and Lorenza, R. (2022). Cabozantinib plus atezolizumab for the treatment of advanced hepatocellular carcinoma: Shedding light on the preclinical rationale and clinical trials. *EXPERT Opin. INVESTIGATIONAL DRUGS* 31 (4), 401–413. doi:10.1080/13543784.2022.2032641

Barroso-Sousa, R., Barry, W. T., Garrido-Castro, A. C., Hodi, F. S., Min, L., Krop, I. E., et al. (2018). Incidence of endocrine dysfunction following the use of different immune checkpoint inhibitor regimens: A systematic review and meta-analysis. *JAMA Oncol.* 4 (2), 173–182. doi:10.1001/jamaoncol.2017.3064

Bertrand, A., Kostine, M., Barnetche, T., Truchetet, M. E., and Schaeverbeke, T. (2015). Immune related adverse events associated with anti-CTLA-4 antibodies:

Systematic review and meta-analysis. *BMC Med.* 13, 211. doi:10.1186/s12916-015-0455-8

Brahmer, J. R., Drake, C. G., Wollner, I., Powderly, J. D., Picus, J., Sharfman, W. H., et al. (2010). Phase I study of single-agent anti-programmed death-1 (MDX-1106) in refractory solid tumors: Safety, clinical activity, pharmacodynamics, and immunologic correlates. *J. Clin. Oncol.* 28 (19), 3167–3175. doi:10.1200/JCO.2009.26.7609

Brahmer, J. R., Lacchetti, C., Schneider, B. J., Atkins, M. B., Brassil, K. J., Caterino, J. M., et al. (2018). Management of immune-related adverse events in patients treated with immune checkpoint inhibitor therapy: American society of clinical oncology clinical practice guideline. *J. Clin. Oncol.* 36 (17), 1714–1768. doi:10.1200/JCO.2017.77.6385

Brahmer, J. R., Tykodi, S. S., Chow, L. Q., Hwu, W. J., Topalian, S. L., Hwu, P., et al. (2012). Safety and activity of anti-PD-L1 antibody in patients with advanced cancer. *N. Engl. J. Med.* 366 (26), 2455–2465. doi:10.1056/NEJMoa1200694

Brown, Z. J., Heinrich, B., Steinberg, S. M., Yu, S. J., and Greten, T. F. (2017). Safety in treatment of hepatocellular carcinoma with immune checkpoint inhibitors as compared to melanoma and non-small cell lung cancer. *J. Immunother. Cancer* 5 (1), 93. doi:10.1186/s40425-017-0298-2

Chen, D. S., and Mellman, I. (2013). Oncology meets immunology: The cancer-immunity cycle. *Immunity* 39, 1–10. doi:10.1016/j.immuni.2013.07.012

Chiew Woon, L., Joycelyn Jie Xin, L., and Su Pin, C. (2020). Nivolumab for the treatment of hepatocellular carcinoma. *Expert Opin. Biol. Ther.* 20 (7), 687–693. doi:10.1080/14712598.2020.1749593

De Martin, E., Michot, J. M., Papouin, B., Champiat, S., Mateus, C., Lambotte, O., et al. (2018). Characterization of liver injury induced by cancer immunotherapy using immune checkpoint inhibitors. *J. Hepatol.* 68 (6), 1181–1190. doi:10.1016/j.jhep.2018.01.033

De Martin, E., Michot, J. M., Rosmorduc, O., Guettier, C., and Samuel, D. (2020). Liver toxicity as a limiting factor to the increasing use of immune checkpoint inhibitors. *JHEP Rep.* 2, 100170. doi:10.1016/j.jhepr.2020.100170

El-Khoueiry, A. B., Sangro, B., Yau, T., Crocenzi, T. S., Kudo, M., Hsu, C., et al. (2017). Nivolumab in patients with advanced hepatocellular carcinoma (CheckMate 040): An open-label, non-comparative, phase 1/2 dose escalation and expansion trial. *Lancet* 389 (10088), 2492–2502. doi:10.1016/S0140-6736(17)31046-2

Finn, R. S., Ikeda, M., Zhu, A. X., Sung, M. W., Baron, A. D., Kudo, M., et al. (2020a). Phase ib study of lenvatinib plus pembrolizumab in patients with unresectable hepatocellular carcinoma. *J. Clin. Oncol.* 38 (26), 2960–2970. doi:10.1200/JCO.20.00808

Finn, R. S., Qin, S., Ikeda, M., Galle, P. R., Ducreux, M., Kim, T. Y., et al. (2020b). Atezolizumab plus bevacizumab in unresectable hepatocellular carcinoma. *N. Engl. J. Med.* 382 (20), 1894–1905. doi:10.1056/NEJMoa1915745

Finn, R. S., Ryoo, B. Y., Merle, P., Kudo, M., Bouattour, M., Lim, H. Y., et al. (2020c). Pembrolizumab as second-line therapy in patients with advanced hepatocellular carcinoma in KEYNOTE-240: A randomized, double-blind, phase III trial. *J. Clin. Oncol.* 38 (3), 193–202. doi:10.1200/JCO.19.01307

Fulgenzi, C., Talbot, T., Murray, S. M., Silletta, M., Vincenzi, B., Cortellini, A., et al. (2021). Immunotherapy in hepatocellular carcinoma. *Curr. Treat. Options Oncol.* 22, 87. doi:10.1007/s11864-021-00886-5

Greten, T. F., Abou-Alfa, G. K., Cheng, A. L., Duffy, A. G., El-Khoueiry, A. B., Finn, R. S., et al. (2021). Society for Immunotherapy of Cancer (SITC) clinical practice guideline on immunotherapy for the treatment of hepatocellular carcinoma. *J. Immunother. Cancer* 9 (9), e002794. doi:10.1136/jitc-2021-002794

Herbst, R. S., Giaccone, G., de Marinis, F., Reinmuth, N., Vergnenegre, A., Barrios, C. H., et al. (2020). Atezolizumab for first-line treatment of PD-L1-selected patients with NSCLC. *N. Engl. J. Med.* 383 (14), 1328–1339. doi:10.1056/NEJMoa1917346

Hodi, F. S., O'Day, S. J., McDermott, D. F., Weber, R. W., Sosman, J. A., Haanen, J. B., et al. (2010). Improved survival with ipilimumab in patients with metastatic melanoma. *N. Engl. J. Med.* 363 (8), 711–723. doi:10.1056/NEJMoa1003466

Huang, S. L., Wang, Y. M., Wang, Q. Y., Feng, G. G., Wu, F. Q., Yang, L. M., et al. (2021). Mechanisms and clinical trials of hepatocellular carcinoma immunotherapy. *Front. Genet.* 12, 691391. doi:10.3389/fgene.2021.691391

Illouz, F., Drui, D., Caron, P., and Do Cao, C. (2018). Expert opinion on thyroid complications in immunotherapy. *Ann. Endocrinol. Paris.* 79, 555–561. doi:10.1016/j.ando.2018.07.007

Kelley, R. K., Rimassa, L., Cheng, A. L., Kaseb, A., Qin, S., Zhu, A. X., et al. (2022). Cabozantinib plus atezolizumab versus sorafenib for advanced hepatocellular carcinoma (COSMIC-312): A multicentre, open-label, randomised, phase 3 trial. *Lancet Oncol.* 23 (8), 995–1008. doi:10.1016/S1470-2045(22)00326-6

Khoja, L., Day, D., Wei-Wu Chen, T., Siu, L. L., and Hansen, A. R. (2017). Tumour- and class-specific patterns of immune-related adverse events of immune checkpoint inhibitors: A systematic review. *Ann. Oncol.* 28 (10), 2377–2385. doi:10.1093/annonc/mdx286

Kurimoto, C., Inaba, H., Ariyasu, H., Iwakura, H., Ueda, Y., Uraki, S., et al. (2020). Predictive and sensitive biomarkers for thyroid dysfunctions during treatment with immune-checkpoint inhibitors. *Cancer Sci.* 111 (5), 1468–1477. doi:10.1111/cas.14363

Larkin, J., Chiarion-Sileni, V., Gonzalez, R., Grob, J. J., Cowey, C. L., Lao, C. D., et al. (2015). Combined nivolumab and ipilimumab or monotherapy in untreated melanoma. *N. Engl. J. Med.* 373 (1), 23–34. doi:10.1056/NEJMoa1504030

Lisi, L., Lacal, P. M., Martire, M., Navarra, P., and Graziani, G. (2022). Clinical experience with CTLA-4 blockade for cancer immunotherapy: From the monospecific monoclonal antibody ipilimumab to probodies and bispecific molecules targeting the tumor microenvironment. *Pharmacol. Res.* 175, 105997. doi:10.1016/j.phrs.2021.105997

Lleo, A., Rimassa, L., and Colombo, M. (2019). Hepatotoxicity of immune check point inhibitors: Approach and management. *Dig. Liver Dis.* 51 (8), 1074–1078. doi:10.1016/j.dld.2019.06.017

Llovet, J. M., Ricci, S., Mazzaferro, V., Hilgard, P., Gane, E., Blanc, J. F., et al. (2008). Sorafenib in advanced hepatocellular carcinoma. *N. Engl. J. Med.* 359 (4), 378–390. doi:10.1056/NEJMoa0708857

Llovet, J. M., Kelley, R. K., Villanueva, A., Singal, A. G., Pikarsky, E., Roayaie, S., et al. (2021b). Hepatocellular carcinoma. *Nat. Rev. Dis. Prim.* 7 (1), 6. doi:10.1038/s41572-020-00240-3

Llovet, J. M., Montal, R., Sia, D., and Finn, R. S. (2018). Molecular therapies and precision medicine for hepatocellular carcinoma. *Nat. Rev. Clin. Oncol.* 15, 599–616. doi:10.1038/s41571-018-0073-4

Llovet, J. M., Villanueva, A., Marrero, J. A., Schwartz, M., Meyer, T., Galle, P. R., et al. (2021a). Trial design and endpoints in hepatocellular carcinoma: AASLD consensus conference. *Hepatology* 73 (1), 158–191. doi:10.1002/hep.31327

Man, S., Luo, C., Yan, M., Zhao, G., Ma, L., and Gao, W. (2021). Treatment for liver cancer: From sorafenib to natural products. *Eur. J. Med. Chem.* 224, 113690. doi:10.1016/j.ejmech.2021.113690

Mazieres, J., Drilon, A., Lusque, A., Mezquita, L., et al. (2019). Immune checkpoint inhibitors for patients with advanced lung cancer and oncogenic driver alterations: Results from the IMMUNOTARGET registry. *Ann. Oncol.* 30 (8), 1321–1328. doi:10.1093/annonc/mdz167

Mitchell, K. A., Kluger, H., Sznol, M., and Hartman, D. J. (2013). Ipilimumab-induced perforating colitis. *J. Clin. Gastroenterol.* 47 (9), 781–785. doi:10.1097/MCG.0b013e31828f1d51

Morganstein, D. L., Lai, Z., Spain, L., Diem, S., Levine, D., Mace, C., et al. (2017). Thyroid abnormalities following the use of cytotoxic T-lymphocyte antigen-4 and programmed death receptor protein-1 inhibitors in the treatment of melanoma. *Clin. Endocrinol. (Oxf)* 86, 614–620. doi:10.1111/cen.13297

Muir, C. A., Clifton-Bligh, R. J., Long, G. V., Scolyer, R. A., Lo, S. N., Carlino, M. S., et al. (2021). Thyroid immune-related adverse events following immune checkpoint inhibitor treatment. *J. Clin. Endocrinol. Metab.* 106, e3704–e3713. doi:10.1210/clinem/dgab263

Nielsen, D. L., Juhl, C. B., Chen, I. M., Kellermann, L., and Nielsen, O. H. (2022). Immune checkpoint inhibitor-induced diarrhea and colitis: Incidence and management. A systematic review and meta-analysis. *Cancer Treat. Rev.* 109, 102440. doi:10.1016/j.ctrv.2022.102440

Pardoll, D. M. (2012). The blockade of immune checkpoints in cancer immunotherapy. *Nat. Rev. Cancer* 12 (4), 252–264. doi:10.1038/nrc3239

Pinato, D. J., Cortellini, A., Sukumaran, A., Cole, T., Pai, M., Habib, N., et al. (2021b). PRIME-HCC: Phase ib study of neoadjuvant ipilimumab and nivolumab prior to liver resection for hepatocellular carcinoma. *BMC Cancer* 21 (1), 301. doi:10.1186/s12885-021-08033-x

Pinato, D. J., Marron, T. U., Mishra-Kalyani, P. S., Gong, Y., Wei, G., Szafron, D., et al. (2021a). Treatment-related toxicity and improved outcome from immunotherapy in hepatocellular cancer: Evidence from an FDA pooled analysis of landmark clinical trials with validation from routine practice. *Eur. J. cancer* 157, 140–152. doi:10.1016/j.ejca.2021.08.020

Rowshanravan, B., Halliday, N., and Sansom, D. M. (2018). CTLA-4: A moving target in immunotherapy. *Blood* 131, 58–67. doi:10.1182/blood-2017-06-741033

Sangro, B., Chan, S. L., Meyer, T., Reig, M., El-Khoueiry, A., and Galle, P. R. (2020). Diagnosis and management of toxicities of immune checkpoint inhibitors in hepatocellular carcinoma. *J. Hepatol.* 72 (2), 320–341. doi:10.1016/j.jhep.2019.10.021

Sangro, B., Gomez-Martin, C., de la Mata, M., Inarrairaegui, M., Garralda, E., Barrera, P., et al. (2013). A clinical trial of CTLA-4 blockade with tremelimumab in patients with hepatocellular carcinoma and chronic hepatitis C. *J. Hepatol.* 59 (1), 81–88. doi:10.1016/j.jhep.2013.02.022

Sangro, B., Sarobe, P., Hervás-Stubbs, S., and Melero, I. (2021). Advances in immunotherapy for hepatocellular carcinoma. *Nat. Rev. Gastroenterol. Hepatol.* 18 (8), 525–543. doi:10.1038/s41575-021-00438-0

Sharma, P., and Allison, J. P. (2015). Immune checkpoint targeting in cancer therapy: Toward combination strategies with curative potential. *Cell* 161, 205–214. doi:10.1016/j.cell.2015.03.030

Sung, H., Ferlay, J., Siegel, R. L., Laversanne, M., Soerjomataram, I., Jemal, A., et al. (2021). Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J. Clin.* 71 (3), 209–249. doi:10.3322/caac.21660

Topalian, S. L., Hodi, F. S., Brahmer, J. R., Gettinger, S. N., Smith, D. C., McDermott, D. F., et al. (2012). Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. *N. Engl. J. Med.* 366 (26), 2443–2454. doi:10.1056/NEJMoa1200690

Villanueva, A. (2019). Hepatocellular carcinoma. *N. Engl. J. Med.* 380 (15), 1450–1462. doi:10.1056/NEJMra1713263

Vogl, T. J., Lammer, J., Lencioni, R., Malagari, K., Watkinson, A., Pilleul, F., et al. (2011). Liver, gastrointestinal, and cardiac toxicity in intermediate hepatocellular carcinoma treated with PRECISION TACE with drug-eluting beads: Results from the PRECISION V randomized trial. *AJR. Am. J. Roentgenol.* 197, W562–W570. doi:10.2214/AJR.10.4379

Wang, D. Y., Salem, J. E., Cohen, J. V., Chandra, S., Menzer, C., Ye, F., et al. (2018). Fatal toxic effects associated with immune checkpoint inhibitors: A systematic review and meta-analysis. *JAMA Oncol.* 4 (12), 1721–1728. doi:10.1001/jamaoncol.2018.3923

Wang, Y., Zhou, S., Yang, F., Qi, X., Wang, X., Guan, X., et al. (2019). Treatment-related adverse events of PD-1 and PD-L1 inhibitors in clinical trials: A systematic review and meta-analysis. *JAMA Oncol.* 5 (7), 1008–1019. doi:10.1001/jamaoncol.2019.0393

Wherry, E. J. (2011). T cell exhaustion. *Nat. Immunol.* 12 (6), 492–499. doi:10.1038/ni.2035

Wong, C. H., Wong, C. S., and Chan, S. L. (2015). Targeting angiogenic genes as a therapeutic approach for hepatocellular carcinoma. *Curr. Gene Ther.* 15 (2), 97–108. doi:10.2174/1566523214666141224094648

Wu, Z., Lai, L., Li, M., Zhang, L., and Zhang, W. (2017). Acute liver failure caused by pembrolizumab in a patient with pulmonary metastatic liver cancer: A case report. *Med. Baltim.* 96 (51), e9431. doi:10.1097/MD.0000000000009431

Xu, F., Jin, T., Zhu, Y., and Dai, C. (2018). Immune checkpoint therapy in liver cancer. *J. Exp. Clin. Cancer Res.* 37, 110. doi:10.1186/s13046-018-0777-4

Yau, T., Kang, Y. K., Kim, T. Y., El-Khoueiry, A. B., Santoro, A., Sangro, B., et al. (2020). Efficacy and safety of nivolumab plus ipilimumab in patients with advanced hepatocellular carcinoma previously treated with sorafenib: The CheckMate 040 randomized clinical trial. *JAMA Oncol.* 6 (11), e204564. doi:10.1001/jamaoncol.2020.4564

Yau, T., Park, J. W., Finn, R. S., Cheng, A. L., Mathurin, P., Edeline, J., et al. (2022). Nivolumab versus sorafenib in advanced hepatocellular carcinoma (CheckMate 459): A randomised, multicentre, open-label, phase 3 trial. *Lancet Oncol.* 23 (1), 77–90. doi:10.1016/S1470-2045(21)00604-5

Younossi, Z., Anstee, Q. M., Marietti, M., Hardy, T., Henry, L., Eslam, M., et al. (2018). Global burden of NAFLD and NASH: Trends, predictions, risk factors and prevention. *Nat. Rev. Gastroenterol. Hepatol.* 15 (1), 11–20. doi:10.1038/nrgastro.2017.109

Zhang, Y., Du, X., Liu, M., Tang, F., Zhang, P., Ai, C., et al. (2019). Hijacking antibody-induced CTLA-4 lysosomal degradation for safer and more effective cancer immunotherapy. *Cell Res.* 29 (8), 609–627. doi:10.1038/s41422-019-0184-1

Zhou, X., Yao, Z., Bai, H., Duan, J., Wang, Z., Wang, X., et al. (2021). Treatment-related adverse events of PD-1 and PD-L1 inhibitor-based combination therapies in clinical trials: A systematic review and meta-analysis. *Lancet Oncol.* 22 (9), 1265–1274. doi:10.1016/S1470-2045(21)00333-8

Zhu, A. X., Finn, R. S., Edeline, J., Cattan, S., Ogasawara, S., Palmer, D., et al. (2018). Pembrolizumab in patients with advanced hepatocellular carcinoma previously treated with sorafenib (KEYNOTE-224): A non-randomised, open-label phase 2 trial. *Lancet Oncol.* 19 (7), 940–952. doi:10.1016/S1470-2045(18)30351-6

Zitvogel, L., Ayyoub, M., Routy, B., and Kroemer, G. (2016a). Microbiome and anticancer immunosurveillance. *Cell* 165 (2), 276–287. doi:10.1016/j.cell.2016.03.001

Zitvogel, L., Pitt, J. M., Daillère, R., Smyth, M. J., and Kroemer, G. (2016b). Mouse models in oncoimmunology. *Nat. Rev. Cancer* 16 (12), 759–773. doi:10.1038/nrc.2016.91

Zongyi, Y., and Xiaowu, L. (2020). Immunotherapy for hepatocellular carcinoma. *Cancer Lett.* 470, 8–17. doi:10.1016/j.canlet.2019.12.002

frontiers | Frontiers in Pharmacology

TYPE Review
PUBLISHED 05 May 2023
DOI 10.3389/fphar.2023.1150151



OPEN ACCESS

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RECEIVED 23 January 2023
ACCEPTED 24 April 2023
PUBLISHED 05 May 2023

CITATION
Sun H, Yang H and Mao Y (2023), Personalized treatment for hepatocellular carcinoma in the era of targeted medicine and bioengineering. *Front. Pharmacol.* 14:1150151. doi: 10.3389/fphar.2023.1150151



# Personalized treatment for hepatocellular carcinoma in the era of targeted medicine and bioengineering

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Hepatocellular carcinoma (HCC) is a major global health burden, causing approximately 8.3 million deaths each year, and it is the third leading cause of cancer-related death worldwide, with a relative 5-year survival rate of around 18%. Due to the advanced stage of diagnosis in most patients, systemic treatment based on targeted therapy has become the only feasible option. Genomic studies have established a profile of molecular alterations in hepatocellular carcinoma with potentially actionable mutations, but these mutations have yet to be translated into clinical practice. The first targeted drug approved for systemic treatment of patients with advanced hepatocellular carcinoma was Sorafenib, which was a milestone. Subsequent clinical trials have identified multiple tyrosine kinase inhibitors, such as Lenvatinib, Cabozantinib, and Regorafenib, for the treatment of hepatocellular carcinoma, with survival benefits for the patient. Ongoing systemic therapy studies and trials include various immune-based combination therapies, with some early results showing promise and potential for new therapy plans. Systemic therapy for hepatocellular carcinoma is complicated by the significant heterogeneity of the disease and its propensity for developing drug resistance. Therefore, it is essential to choose a better, individualized treatment plan to benefit patients. Preclinical models capable of preserving *in vivo* tumor characteristics are urgently needed to circumvent heterogeneity and overcome drug resistance. In this review, we summarize current approaches to targeted therapy for HCC patients and the establishment of several patient-derived preclinical models of hepatocellular carcinoma. We also discuss the challenges and opportunities of targeted therapy for hepatocellular carcinoma and how to achieve personalized treatment with the continuous development of targeted therapies and bioengineering technologies.

KEYWORDS

**hepatocellular carcinoma, target therapy, personalized therapy, bioengineering technolog, precision medicine**

## 1 Introduction

Worldwide, hepatocellular carcinoma (HCC) remains the fourth most common cause of cancer-related death, and its global burden continues to increase each year (Villanueva, 2019; Llovet et al., 2021; Vogel et al., 2022). With the development of diagnosis and treatment technologies in recent decades, the treatment of HCC has progressed greatly. However, except for cases detected early, most patients are currently diagnosed at a later stage and curative treatments are frequently not available (Benson et al., 2021; Llovet et al., 2021; Vogel

et al., 2022). Therefore, systemic therapies (combination therapies) are the key to the survival of advanced HCC patients (Gordan et al., 2020). Targeted therapy and immunotherapy are the most studied and applied systemic treatment methods in recent years, and they are playing an increasingly important part in the treatment of patients with advanced HCC (Llovet et al., 2018; Huang et al., 2020). In order to enhance the survival rate of HCC patients, precise and individualized treatments will become the future of HCC systemic treatment.

The extensive intratumoral heterogeneity of HCC and the non-negligible drug resistance of targeted drugs are the main obstacles for developing individualized HCC treatments (Fisher et al., 2013; Schulze et al., 2015; Zucman-Rossi et al., 2015; Mcgranahan and Swanton, 2017). In the past, traditional tumor models could not reflect the heterogeneity of different HCC patients nor could they be used for research on targeted drug resistance in different patients hindering the development of personalized treatment for HCC. With the development of bioengineering techniques in recent years, patient-derived liver cancer preclinical models reflecting the complex characteristics of tumors can now be created, showing great promise to benefit the development of personalized medicine for HCC patients and improve clinical outcomes (Bresnahan et al., 2020).

In this review, we examine recent advances in targeted therapies for liver cancer and discuss the application of bioengineered models of liver cancer to personalized treatment of liver cancer, including the novel clinical trials and technology platforms expected to facilitate substantial progress over the next decade.

# 2 Targeted therapy

Targeted therapy embodies the precise treatment for HCC. With the completion of the Genome Project, the molecular alteration profile of HCC is well known (Craig et al., 2020; Rebouissou and Nault, 2020). Numerous studies demonstrated genes from multiple signaling pathways, such as Wnt/β-catenin, P53/cell cycle regulation, oxidative stress, epigenetic modifiers, et al., were frequently mutated in HCC (Boyault et al., 2007; Hoshida et al., 2009; Schulze et al., 2015). Molecularly targeted drugs modulating these molecules and pathways have become a hot area in liver cancer research, but only a small number of tumors, about 25%, have potentially targetable drivers (Schulze et al., 2015). Therefore, it is extremely difficult to develop effective therapies other than surgery for HCC. Similarly, various cytokines involved in these signaling pathways, such as vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), transforming growth factor-α (TGF-α), and insulin-like growth factor-II have also been extensively studied. Among them, anti-angiogenic drugs targeting the VEGF play an important role in the targeted therapy of HCC (Weis and Cheresh, 2011).

## 2.1 Sorafenib

The groundbreaking study (SHARP) in 2007 demonstrated that the tyrosine kinase inhibitor (TKI) sorafenib resulted in a 3-month overall survival (OS) benefit to patients with advanced liver cancer (Llovet et al., 2008; 2018). Subsequent clinical trials also proved the effectiveness of sorafenib in improving OS and objective response rate (ORR). Sorafenib was the only therapy with confirmed survival benefits for patients with advanced liver cancer for a long time thereafter (Cheng et al., 2009). Although the efficacy and safety of sorafenib have brought improvements to the treatment of HCC, (Bruix et al., 2012; Raoul et al., 2012; EASL Clinical Practice Guidelines and European Association for the Study of the Liver, 2018), the average survival time of patients is still less than 1 year. Therefore, some studies have turned their attention to combination therapy. The existing clinical trials proved that in the treatment of advanced HCC, sorafenib combined with Transarterial chemoembolization (TACE), external irradiation, and other therapies prolong the disease-free survival time and OS time compared with sorafenib alone (Qu et al., 2012; Meyer et al., 2017; Zhao et al., 2019). In recent years, targeted therapies have continued to develop. Currently, there are first-line TKIs lenvatinib and donafenib, as well as second-line treatments regorafenib, cabozantinib and apatinib, which have been proven to be effective for advanced liver cancer (Table 1).

## 2.2 Lenvatinib

Compared with sorafenib, lenvatinib has advantages in reducing drug resistance. Drug resistance caused by long-term sorafenib treatment of liver cancer is one of the leading clinical problems at present. Sorafenib resistance is generally believed to be due to the presence of FGF, a pro-angiogenic factor thought to increase tumor cell resistance to anti-VEGF therapy (Tang et al., 2020). As a TKI, lenvatinib can selectively inhibit VEGF receptors (VEGFR) 1-3, FGF receptors 1-4, platelet-derived growth factor receptor-α, RET, and cKIT, which may reduce the occurrence of drug resistance during treatment (Al-Salama et al., 2019). The results of the REFLECT trial in 2018 showed that in the treatment of advanced HCC, the inhibitory effect of lenvatinib on tumors was not inferior to that of sorafenib (Kudo et al., 2018). Lenvatinib especially improved the efficacy of secondary endpoints, compared with sorafenib and it significantly prolonged the median progression-free time and improved the ORR. The curative effect of lenvatinib on patients with hepatitis B virus-related HCC is better than that of sorafenib (Al-Salama et al., 2019). Lenvatinib is the second first-line drug for advanced HCC and was developed 10 years after sorafenib (Hiraoka et al., 2019). It not only provides new drug options for patients with advanced liver cancer but also provides evidence for subsequent drug development.

## 2.3 Regorafenib

The current second-line molecular targeted drugs, regorafenib and cabozantinib, can be used as monotherapy for HCC patients who have progressed on sorafenib. Regorafenib was approved as the first second-line treatment for HCC patients who progressed during or after sorafenib treatment and ushered in the era of second-line and sequential therapy (Finn et al., 2018). Regorafenib is a multi-target TKI discovered during the process of adjusting the molecular structure of sorafenib to

**TABLE 1 Approved clinical trials of targeted therapy for advanced HCC.**

| Name/identifier | Study type | Drug | Target | Line |
|---|---|---|---|---|
| SHARP/NCT00105443 | Phase III | Sorafenib | Multikinases | First-line |
| REFLECT/NCT01761266 | Phase III | Lenvatinib | Multikinases | First-line |
| RESORCE/NCT01774344 | Phase III | Regorafenib | Multikinases | Second-line |
| CELESTIAL/NCT01908426 | Phase III | Cabozantinib | Multikinases | Second-line |
| REACH-2/NCT02435433 | Phase III | Ramucirumab | VEGFR2 | Second-line |
| AHELP/NCT02329860 | Phase III | Apatinib | Multikinases | Second-line |

optimize its curative effect. It has a stronger effect antagonizing VEGFR kinase and inhibiting TIE2, cKIT, and RET kinases. The results of the RESORCE trial showed that for HCC patients whose tumors continued to progress during sorafenib monotherapy, regorafenib could significantly prolong the patient's OS (2.8 months), progression-free survival (PFS), and time to progression (TTP) which were significantly longer than those of the placebo group (Bruix et al., 2017). Subsequent relevant clinical studies have also confirmed the effectiveness of regorafenib, and sequential treatment with sorafenib has been shown to be effective for patients with HCC recurrence after liver transplantation (Iavarone et al., 2019; Yoo et al., 2019).

## 2.4 Cabozantinib

Cabozantinib is a TKI targeting kinases such as MET, AXL, and VEGFR1-3. It is also used as a second-line drug for advanced liver cancer that is not responsive to sorafenib treatment. The CELESTIAL Phase 3 clinical trials showed that cabozantinib can significantly prolong the OS of patients (2.2 months) and the ORR and PFS were not significantly different from the RESORCE trial of regorafenib (Abou-Alfa et al., 2018). Cabozantinib, which differs from regorafenib, can be used in patients who are intolerant to sorafenib. However, cabozantinib treatment may have more toxic side effects than regorafenib (Kudo, 2018). The results of the cost-effectiveness analysis also showed that its economic cost is higher than that of regorafenib, although sorafenib-resistant HCC patients can benefit from cabozantinib treatment (Parikh et al., 2017; Soto-Perez-De-Celis et al., 2019). Therefore, choosing the right patient population is very important.

## 2.5 Ramucirumab

Unlike the above-mentioned second-line TKI drugs, ramucirumab is a recombinant monoclonal antibody targeting VEGFR2, which can block the ligand-receptor interaction and its downstream signaling to exert anti-tumor effects (Syed, 2020). Ramucirumab failed to meet its primary endpoint as second-line therapy in the REACH trial and ramucirumab did not significantly improve OS compared with placebo (9.2 months vs. 7.6 months) (Zhu et al., 2015). However, subgroup analysis confirmed that patients with elevated AFP (≥400 ng/mL) could obtain a better survival benefit from ramucirumab treatment (Chau et al., 2017; Zhu et al., 2017; Gilabert and Raoul, 2018). This was subsequently confirmed by the REACH-2 trial, (Zhu et al., 2019), and ramucirumab was the first FDA-approved drug for HCC patients in a biomarker-selected group (AFP ≥400 ng/mL), but its underlying biomarker-driven mechanism still needs to be further explored (Montal et al., 2019; Zhu et al., 2021).

## 2.6 Immune checkpoint inhibitors

In addition to molecularly targeted therapy, immunotherapy is becoming another clinical weapon for the systemic treatment of liver cancer. Immune evasion in HCC is an important cause of tumor progression. Immune checkpoint proteins are glycoproteins on the cell surface that transmit inhibitory signals to T cells and natural killer cells, and are widely expressed on tumor cells, macrophages, dendritic cells, and other immune cells. These proteins inhibit the excessively activated T cell response, thereby acting on the tumor-immune tolerance process. Tumor cells can inhibit the activation of T cells by expressing immune checkpoint-related molecules and escape the body's immune surveillance (Llovet et al., 2022). In HCC, anti-PD-1 monoclonal antibodies and anti-PD-L1 monoclonal antibodies are currently the most studied, clinically meaningful immune checkpoint inhibitors (ICIs).

As more clinical trials are completed, the number of first- or second-line drugs for immunotherapy also increases. Currently, immune drugs approved for the first-line treatment include atezolizumab, sindilizumab, camrelizumab, and pembrolizumab. The combination of these drugs or with anti-angiogenic molecularly targeted drugs, for example, the combination of atezolizumab and bevacizumab, (Finn et al., 2020), sintilizumab and bevacizumab biosimilar IBI305, (Ren et al., 2021), camrelizumab Mab plus apatinib, (Xu et al., 2021), or pembrolizumab plus lenvatinib, (Ikeda et al., 2019), were promising in completed clinical trials.

The immune drugs that have been approved for second-line treatment include camrelizumab, pembrolizumab, nivolumab, and ipilimumab. Treatment options include immune monotherapy, immunotherapy combined with anti-angiogenic targeted therapy, and combined immunotherapies (El-Khoueiry et al., 2017; Qin et al., 2020; Qin et al., 2022). Overall, combination therapy including immunotherapy significantly improved ORRs and prolonged OS compared with immunotherapy alone (Table 2).

**TABLE 2 Clinical trials of combination therapy for advanced HCC.**

| Name/identifier | Study type | Drug | Target | Primary endpoint | Line |
|---|---|---|---|---|---|
| IMbrave150/NCT03434379 | Phase III | Atezolizumab Bevacizumab | PD-L1 | 19.2 months (mOS) | First-line treatment |
| | | | VEGF | 29.8 (ORR) | |
| NCT03794440 | Phase II/III | Sintilizumab IBI305 | PD-1 | — | — |
| | | | VEGF | | |
| RESCUE/NCT03463876 | Phase II | Camrelizumab Apatinib | PD-1 | — | — |
| NCT03764293 | Phase III | | VEGFR2 | — | — |
| KEYNOTE524/NCT03006926 | Phase Ib | Lenvatinib Pembrolizumab | Multikinases | 9.3 months (mPFS) | — |
| | | | PD-1 | 22 months (mOS) | |
| | | | | 46% (ORR) | |
| LEAP-002/NCT03713593 | Phase III | | | — | — |

# 3 Challenges and opportunities of targeted therapy for HCC

## 3.1 Challenges

As mentioned above, the occurrence and development of HCC is a complex multi-pathway-mediated process. The emergence of the aforementioned targeted drugs has given people more confidence in the future treatment of HCC. New drugs for targeted therapy are also being continuously developed.

Because of the complexity of HCC, monotherapy often leads to dose- or time-dependent severe adverse events (AEs), resulting in treatment interruption due to intolerance. Consequently, the efficacy of single drugs such as TKI or ICI has reached a bottleneck at an OS of 14–16 months. This also suggests that the developmental process of targeted drugs should be changed. Therefore, in the past 2 years, various combinations of ICI and anti-VEGF monoclonal antibodies have been the research focus of HCC-targeted therapy, which has greatly improved the survival rate of advanced HCC patients and created a new combination for targeted therapy (Ouyang et al., 2022).

As mentioned above, for the first-line immunotherapy regimen, the combination of ICIs and anti-VEGF monoclonal antibodies (atezolizumab combined with bevacizumab), has better clinical outcomes than sorafenib and there is no significant difference in the risk of AEs (Finn et al., 2020). The phase II study (RESCUE) of the novel VEGFR2 TKI apatinib in combination with camrelizumab showed an exciting survival benefit and safety profile. An ongoing randomized, open-label, multicenter, phase III trial (NCT03764293) comparing this combination with sorafenib in advanced HCC is promising; however, combination did not meet the expected endpoint. The double-blind, randomized controlled phase III LEAP-002 trial of lenvatinib plus placebo, which completed recently, showed the median OS period of lenvatinib and pembrolizumab was 21.2 months but did not meet its prespecified co-endpoints of significantly improved OS or PFS (Finn Rs, 2022). In conclusion, various combinations of ICIs and anti-angiogenic drugs significantly improved the clinical survival of advanced HCC patients. The clinical applicability of combined targeting is promising, but at the same time, the accompanying risk of AEs cannot be ignored.

## 3.2 Opportunities

The continuous emergence of the above-mentioned targeted drugs and combined treatment options in recent years increased confidence in the treatment of HCC. Nevertheless, it cannot be ignored that HCC is a type of solid tumor with a complex tumor microenvironment consisting of various liver non-parenchymal cells, extracellular matrix proteins, and signaling molecules, which play an important role in tumor evolution and response to treatment by inducing inflammation, angiogenesis, hypoxia, and fibrosis. Drug resistance in HCC is closely related to its tumor heterogeneity and evolution, and drug resistance is also the main reason for targeted therapy treatment failure. Therefore, in the individualized treatment of HCC, avoiding tumor heterogeneity and mastering tumor evolution to overcome drug resistance are the key points to achieving breakthrough progress. In short, identifying specific patient populations that respond to individual treatments and finding clear drug-sensitivity markers are clinical problems that urgently need to be solved for precise targeted therapy.

The rapid development of next-generation sequencing technology (NGS) provides new hope for the precision of targeted therapy (Collins and Varmus, 2015). Biomarker-driven targeted therapy can be adjusted and customized individually by NGS. Therefore, NGS can improve the ability to differentiate individual characteristics of tumors and has the potential to identify new therapeutic targets, thereby ushering in the era of precision medicine (Collins and Varmus, 2015; Karlovich and Williams, 2019). But much remains to be done to successfully bring NGS closer to impacting clinical care in HCC.

Several studies have shown the value of NGS in precisely targeted therapy of HCC. Utilizing archived tumor tissue and baseline plasma samples from HCC patients in the RESORCE trial of regorafenib, a plasma miRNA panel and gene mutation signature in tumors were found to predict response to regorafenib

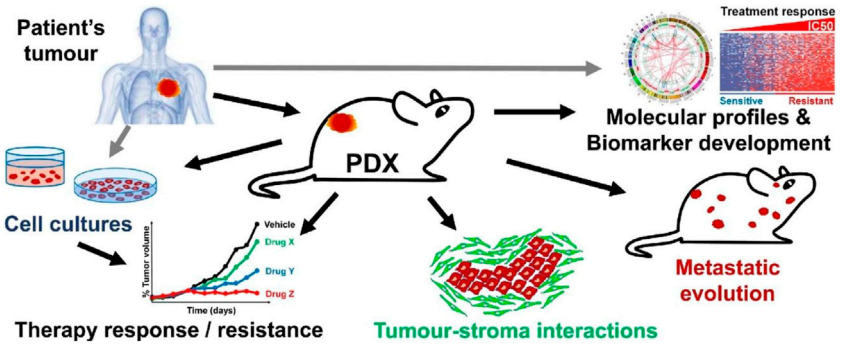


FIGURE 1
Generation and application of PDX. Patient-derived tumors are implanted in mice, and after the implantation phase, it is transplanted and expanded to generate PDX cohorts suitable for preclinical studies, including drug testing and molecular profiling studies. In addition, PDX-derived tumor samples can be collected to create tissue biobanks, which have important implications for sustainable preclinical research, his figure was cited from Invrea, et al. (2020). Note: This is an open access article distributed under the Creative Commons Attribution License that permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited (CC BY 4.0).

(Teufel et al., 2019). In BIOSTORM, STORM's biomarker companion study identified polygenic signatures related to improved relapse-free survival (RFS) with sorafenib adjuvant therapy after hepatectomy, which could be used to guide treatment options in the future (Pinyol et al., 2019). In addition, NGS is also a key tool for developing potential drug-sensitivity markers for targeted therapies. Using the FDA-approved 468-genome MSK-IMPACT, Harding et al (Harding et al., 2019) were able to prospectively identify mutations that were predictive of adverse outcomes with sorafenib and ICIs.

Important advances have been made in lung and colorectal cancers by selecting patients for treatment based on their molecular characteristics, whereas HCC is limited by complex significant molecular heterogeneity. But the improved resolution of NGS technology enables the discovery of significant tumor heterogeneity. With an appropriate and well-designed protocol, the prediction of a potential biomarker response to a specifically targeted drug is greatly increased. In recent years, the field of artificial intelligence (AI) has experienced rapid growth, driven by the development of big data models and deep learning algorithms. This technology has shown great potential for predicting targeted therapy biomarkers and managing the prognosis of hepatocellular carcinoma (Chen et al., 2020; Ahn et al., 2021; Zeng et al., 2022). Therefore, the combination of AI with NGS is expected to further enhance the accuracy of precision medicine for hepatocellular carcinoma.

In addition to identifying response biomarkers by NGS, establishing individual drug screening platforms is an urgent need to overcome drug resistance. Fortunately, the rapid development of bioengineering technology has made it possible to develop excellent HCC preclinical models to track tumor evolution and study drug resistance mechanisms. At present, patient-derived xenograft (PDX) and patient-derived organoid (PDO) models are widely used, which can simulate the occurrence of liver cancer and the tumor microenvironment and provide a preclinical platform for drug screening, biomarker development, drug resistance changes, and mechanism research. Additionally, the rapid development of three dimensional (3D) biology in recent years has provided more possibilities for the development of HCC preclinical models.

# 4 Bioengineering

## 4.1 Patient-derived xenografts

The PDX model first appeared more than 50 years ago and was applied to the research of colorectal cancer (Rygaard and Povlsen, 1969). In 1996, the HCC PDX model was established for the first time (Sun et al., 1996). The subsequent HCC PDX development was slow, cumbersome, and inefficient. However, clinical research results in recent years stimulated hope for the application of PDX models in liver cancer research. The HCC PDX model is currently a mature and ideal tumor model for HCC, which accurately recapitulates the genetic complexity of human tumors, mimics the *in vivo* interactions of tumors with their surrounding tissues and has good clinical predictability (Figure 1); (Brown et al., 2018; Invrea, et al., 2020)

One of the key points in establishing HCC PDX models is the selection of animals for transplantation. Another key point is the selection of the injection site. Subcutaneous injection of cells/tumor (heterotopic model) is the simplest *in vivo* method, which can more accurately measure tumor growth and response to treatment (Brown et al., 2018). However, this approach results in subcutaneously transplanted tumors lacking a tumor-associated microenvironment. Orthotopic transplantation can provide a microenvironment similar to that of the matched tumor tissue and has a rich blood supply, which greatly retains the specificity and microenvironmental characteristics of the patient's tumor (Hernandez-Gea et al., 2013). It is currently the most ideal transplantation method, but compared with heterotopic transplantation, its technical difficulty, low success rate, cumbersome tumor assessment, and other issues affect its expanded application.

Currently, the most commonly used model is a xenograft model implanting patient-derived samples in an immunodeficient mouse

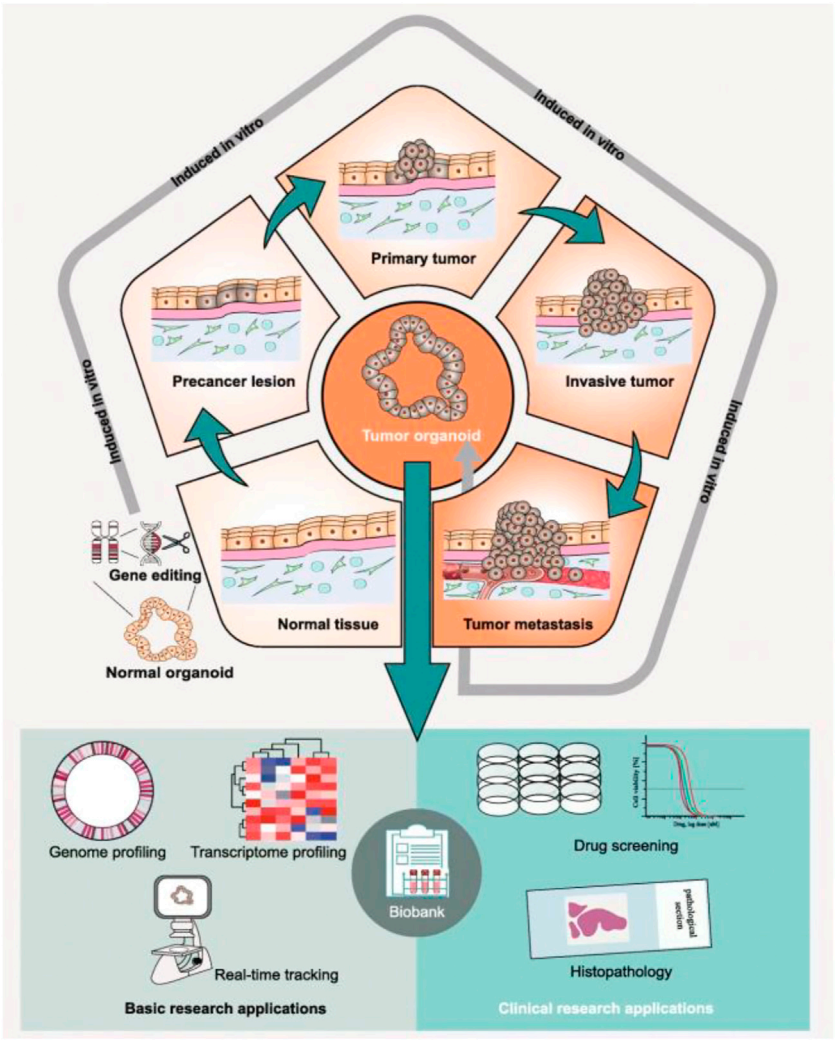


FIGURE 2
Preclinical applications of patient-derived tumor organoids. Whether in preclinical basic research or clinical research, patient-derived tumor organoids have great application value. This figure was cited from Fan, et al. (2019). Note: This is an open access article distributed under the Creative Commons Attribution License that permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited (CC BY 4.0).

to prevent the mouse's immune system from rejecting the human tumor. However, this PDX model in immunodeficient mice cannot be used to study immunotherapeutic approaches. Therefore, recapitulating the complexity of the human immune system in preclinical models is critical for studying immunity and immunotherapy in HCC. One strategy to achieve this goal is to use humanized mouse models modified to contain human immune cells (Brown et al., 2018; Zhao et al., 2018). There are different approaches to humanizing the mouse immune system. Even so, a lot of work is needed to fully personalize humanized mice to match each patient sample.

PDXs are already a mature and excellent individualized treatment platform in the preclinical model of HCC, and have shown advantages as a preclinical model in mechanism research, drug and biomarker screening, and combined clinical trials (Hu et al., 2019). PDXliver is the first public database of liver cancer PDX models, including drug response data, which fully reflects the heterogeneity of HCC and is conducive to the discovery of biomarkers for specific treatments (He et al., 2018). Recently, Jin et al. (2021) found that lenvatinib and gefitinib had antitumor activity in HCC PDX with high expression of epidermal growth factor receptor (EGFR), and EGFR has been used as a biomarker to achieve a more informed patient stratification in clinical trials. The HCC PDX model has proven valuable in exploring many different aspects of precision oncology in preclinical research. However, the time commitment, low success rate, and large resource requirement of PDX also limit its wide application.

## 4.2 Patient-derived organoids

To circumvent the limitations of two dimensional (2D), monolayer cell line tumor models, researchers have been trying to grow tumors in 3D for a long time using methods, such as liver

slices and mechanical 3D culture devices (Tharehalli et al., 2019). However, these models fall short in terms of culture time and preservation of the original tumor characteristics. One of the main purposes of 3D tumor cell culture is to simulate the growth environment of tumors in the human body and to preserve the genetic and histological characteristics of the parent tumor to the greatest extent. Only such preclinical models can be applied to individualized treatment.

In 2012, the first tumor organoids were developed, derived from intestinal tumors (Sato et al., 2011). Tumor organoids have given rise to a new concept of 3D culture, characterized by self-organized 3D structures that mimic the original *in vivo* structure of an organ or tumor and can be obtained from different sources (Figure 2); (Fan, et al., 2019; Tuveson and Clevers, 2019) Thus far, organoids have been derived from organ-specific adult stem cells, pluripotent stem cells (PSCs), embryonic stem cells, or induced pluripotent stem cells (iPSCs), as well as tumors. Huch et al. (2015) established the first liver organoids from mice and human liver stem cell organoids, in 2013 and 2015, respectively, in which stem cells can be expanded for a long time and differentiated into biliary or hepatic cells according to the composition of the medium. In addition, tissue-specific organoids can be established using PSCs. Takebe et al. (2013) constructed liver organoids from human iPSCs combined with endothelial and mesenchymal cells in Matrigel. At present, liver multicellular co-culture organoids have been reported by many studies, including a mixed culture of hepatocytes and various mesenchymal cells and iPSC-derived liver organoids on perfusion microcolumn chips.

The application of the above healthy liver organoids in tumor therapy is mainly used to the study carcinogenesis, such as the carcinogenesis induced by the hepatitis B virus. Of course, organoids from patient-derived liver tumors are the most direct and effective method to study the individualized treatment of tumors. There are two main sources of PDOs, needle biopsy and surgically obtained human tumor specimens. Due to the difficulty and complexity of organoid culture, both routes are less effective in establishing HCC organoids (37.5% and 26%, respectively) (Broutier et al., 2017; Nuciforo et al., 2018). However, even at low tumor to stromal cell ratios, there are opportunities to establish PDO. This helps avoid short comings of NGS. Furthermore, it is more important that HCC PDOs are highly concordant with original tumor biopsies in terms of growth pattern, degree of differentiation, expression of HCC-specific markers, genomic alterations, and ability to form tumors in xenograft models (Broutier et al., 2017; Nuciforo et al., 2018). This makes PDOs more suitable for precision medicine, including targeted therapy resistance research, drug screening, and treatment response prediction. Li et al. established HCC organoids for drug screening using surgical specimens of primary human liver cancer, and proved that PDOs can be used as preclinical models for the individualized treatment of HCC (Li et al., 2019). By establishing HCC PDOs, Wang et al. (2020); Leung et al. (2020) found reactivation of Hedgehog signaling and receptor tyrosine kinase-induced MEK/ERK and AKT signaling pathways may be related to sorafenib resistance in HCC. In addition to Matrigel encapsulation, a recent study used hydrogel capsules to culture HCC PDOs to simulate the tumor microenvironment of liver cancer, and demonstrated the heterogeneity of the platform for targeted drugs and other applications that can be used to assist individualized therapy (Dong et al., 2022). Clinical trials (NCT05384184 and NCT02436564) examining HCC PDOs are already underway.

The ongoing development of HCC PDOs will be focused on better simulating the tumor microenvironment *in vivo*. Loh et al. (2021) constructed a HCC PDO model and explored signaling axes that enhance hepatocyte resistance to sorafenib by culturing organoids in conditioned medium to mimic the tumor microenvironment. Recently, Lim et al. (2022) used a hydrogel system to establish a co-culture model to mimic and characterize pro-angiogenic secretory signaling between hepatoma cells and endothelial cells *in vitro*. A PDO-TME model combining PDOs and the tumor microenvironment cells will provide a more accurate platform for liver cancer-targeted therapy to avoid heterogeneity and overcome drug resistance research, and it will also be more conducive to the development of new drugs.

## 4.3 3D bioprinting of HCC

In recent years, additive manufacturing, or 3D printing, has been applied to biomedicine, called 3D bioprinting (3DP). The development of 3DP has opened a new chapter in bioengineered medicine (Murphy and Atala, 2014). 3DP is mainly achieved through inkjet, micro-extrusion, or laser-assisted bioprinting, among which micro-extrusion is the most widely used (Mandrycky et al., 2016; Matai et al., 2020). The core material of 3DP is bio-ink and its potential for clinical translation depends on the formulation of the bio-ink (Gu et al., 2022). Bio-inks that have been extensively studied include alginate, fibrinogen, gelatin, collagen, chitosan, agarose, Pluronic, hyaluronic acid, GelMA, PEG, and decellularized extracellular matrix. The choice of bio-ink is also based on the choice of bioprinting technology (Gu et al., 2022).

3DP has been widely used in cancer research (Almela et al., 2018; Wang et al., 2018; Swaminathan et al., 2019; Sbirkov et al., 2021; Xie et al., 2021). 3DP tumor models can provide physiologically relevant cell-cell and cell-matrix interactions by mimicking the 3D heterogeneity of real tumors (Figure 3); (Knowlton et al., 2015; Augustine et al., 2021; Jung, et al., 2022) We initially created a 3DP model of HepG2 cells by 3DP technology based on a gelatin-sodium alginate bio-ink system (Sun et al., 2020). We found that compared with 2D-HepG2 cells, the 3DP-HepG2 model displayed significantly increased levels of various liver function-related proteins and genes as well as those involved in proliferation, metastasis, drug resistance, anti-tumor immunosuppression, and tumor cell epithelial-mesenchymal transition. This gives the 3DP tumor model unique advantages in the preclinical research of liver cancer and the 3DP tumor model can be used as a more suitable platform for anti-tumor drug development. Next, we have previously revealed that patient-derived primary hepatocellular carcinoma cells could maintain high activity long-term in gelatin-sodium alginate bio-ink-based 3DP model and could be used for liver cancer-targeted therapy drug testing for the prediction of personalized therapy (Xie et al., 2021). We found that 3DP tumor models offer advantages in terms of cost, modeling success rate, maintenance of cell viability, establishment success rate, and low time commitment. 3DP printing relies on a computer to generate the design, high precision, and repeatability of the tumor model, which has inherent advantages over the PDX and

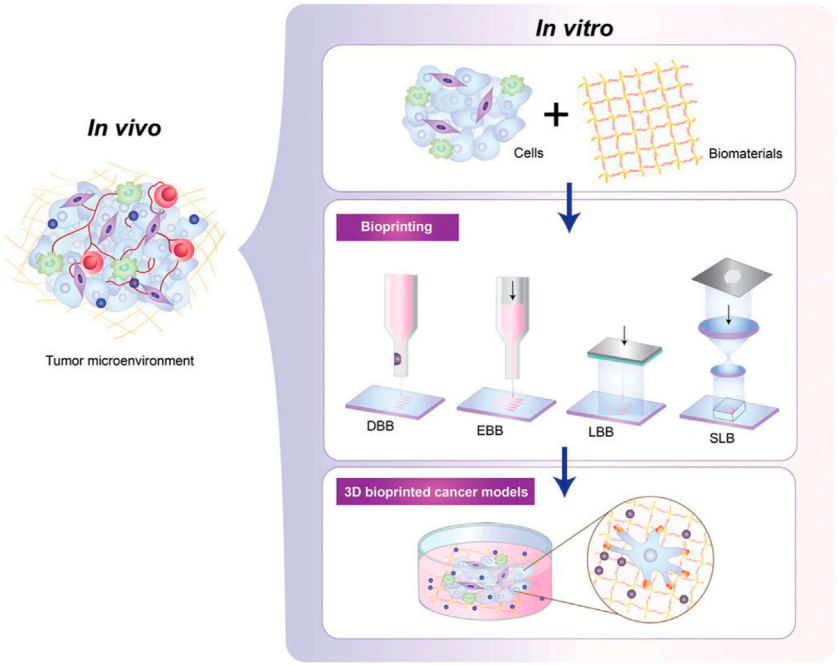


FIGURE 3
The development process of patient-derived tumor 3DP models. Tumor cells are wrapped in specific bio-inks with high biocompatibility, and then 3DP tumor models can be generated in different ways in high-precision bioprinters, including droplet-based (DBB), extrusion-based (EBB), laser -based (LBB) and stereolithography (SLB) bioprinting. This figure was cited from Jung, et al. (2022). Note: This is an open access article distributed under the Creative Commons Attribution License that permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited (CC BY 4.0).

**TABLE 3 Relative advantages related to PDX and PDO.**

| | PDX | PDO | 3DP |
|---|---|---|---|
| Establishment success rate | – | – | ++ |
| Cost | – | + | ++ |
| Recapitulates tumoural heterogeneity | + | + | + |
| Multi-Cell culture conditions | + | + | ++ |
| Microenvironment construction | ++ | + | ++ |
| High-throughput screening | – | + | + |

PDO models. In addition, if a technological breakthrough in the rapid expansion of primary liver cancer cells can be achieved, 3D bioprinting will play a greater role in the application of liver cancer *in vitro* models.

3DP cancer models can recapitulate a tumor's microstructure and function and preserve the parental tumor's features. Therefore, it is an ideal 3D, preclinical model with a higher success rate in construction and drug testing than existing traditional cancer models (Table 3); (Shukla et al., 2022) In addition, the absolute advantage of 3DP models as preclinical models is that printers can enable the fabrication of high-resolution microstructures to reproduce the complexity of the tumor microenvironment, including the vascularization of tumors (Shukla et al., 2022). At present, bioprinting has been applied in 3DP models of breast cancer and glioblastoma (Zhou et al., 2016; Heinrich et al., 2019). The application of a 3DP-TME cancer model combining 3DP HCC tumors and the tumor microenvironment needs to be verified by additional research.

3DP can also be combined with cancer chip technology to reproduce key tumor microenvironmental characteristics (Monteiro et al., 2022; Shukla et al., 2022) and generate an increasing number of biomimetic tumor models for precision and personalized medicine, which is of great significance for studying HCC drug resistance and tumor evolution *in vitro*.

## 5 Conclusion

The systemic treatment of HCC has made considerable progress, and a higher number of treatment options are now available. However, due to the huge heterogeneity of HCC, the focus of HCC research should not only be on drug development but also on how to accurately select individualized treatment options. This will save more advanced patients from the toxic side effects of drugs with no tumor response and increase patient survival time. The development of bioengineered models of tumors shows great promise for personalized medicine and improved HCC outcomes. At present, there are relatively few clinical studies, and more studies using clinical specimens are needed to clarify whether the bioengineered model of HCC is suitable for capturing intratumoral heterogeneity and predicting patient response to treatments such as targeted therapy.

## Author contributions

YM, HY, and HS conceived of designed, and supervised the study. HS contributed to writing of the manuscript. YM and HY contributed to review and revision of the manuscript.

## Funding

This work was supported by the CAMS Innovation Fund for Medical Sciences (2021-I2M-1-058, 2022-I2M-2-003), National High Level Hospital Clinical Research Funding (2022-PUMCH-B-034) and National Natural Science Foundation of China (32271470).

## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

## Publisher's note



## References

Abou-Alfa, G. K., Meyer, T., Cheng, A. L., El-Khoueiry, A. B., Rimassa, L., Ryoo, B. Y., et al. (2018). Cabozantinib in patients with advanced and progressing hepatocellular carcinoma. *N. Engl. J. Med.* 379 (1), 54–63. doi:10.1056/NEJMoa1717002

Ahn, J. C., Qureshi, T. A., Singal, A. G., Li, D., and Yang, J. D. (2021). Deep learning in hepatocellular carcinoma: Current status and future perspectives. *World J. Hepatol.* 13 (12), 2039–2051. doi:10.4254/wjh.v13.i12.2039

Al-Salama, Z. T., Syed, Y. Y., and Scott, L. J. (2019). Lenvatinib: A review in hepatocellular carcinoma. *Drugs* 79 (6), 665–674. doi:10.1007/s40265-019-01116-x

Almela, T., Al-Sahaf, S., Brook, I. M., Khoshroo, K., Rasoulianboroujeni, M., Fahimipour, F., et al. (2018). 3D printed tissue engineered model for bone invasion of oral cancer. *Tissue Cell.* 52, 71–77. doi:10.1016/j.tice.2018.03.009

Augustine, R., Kalva, S. N., Ahmad, R., Zahid, A. A., Hasan, S., Nayeem, A., et al. (2021). 3D Bioprinted cancer models: Revolutionizing personalized cancer therapy. *Transl. Oncol.* 14 (4), 101015. doi:10.1016/j.tranon.2021.101015

Benson, A. B., D'Angelica, M. I., Abbott, D. E., Anaya, D. A., Anders, R., Are, C., et al. (2021). Hepatobiliary cancers, v ersion 2.2021, NCCN clinical Practice Guidelines in oncology. *J. Natl. Compr. Canc Netw.* 19 (5), 541–565. doi:10.6004/jnccn.2021.0022

Boyault, S., Rickman, D. S., de Reynies, A., Balabaud, C., Rebouissou, S., Jeannot, E., et al. (2007). Transcriptome classification of HCC is related to gene alterations and to new therapeutic targets. *Hepatology* 45 (1), 42–52. doi:10.1002/hep.21467

Bresnahan, E., Ramadori, P., Heikenwalder, M., Zender, L., and Lujambio, A. (2020). Novel patient-derived preclinical models of liver cancer. *J. Hepatol.* 72 (2), 239–249. doi:10.1016/j.jhep.2019.09.028

Broutier, L., Mastrogiovanni, G., Verstegen, M. M., Francies, H. E., Gavarro, L. M., Bradshaw, C. R., et al. (2017). Human primary liver cancer-derived organoid cultures for disease modeling and drug screening. *Nat. Med.* 23 (12), 1424–1435. doi:10.1038/nm.4438

Brown, Z. J., Heinrich, B., and Greten, T. F. (2018). Mouse models of hepatocellular carcinoma: An overview and highlights for immunotherapy research. *Nat. Rev. Gastroenterol. Hepatol.* 15 (9), 536–554. doi:10.1038/s41575-018-0033-6

Bruix, J., Qin, S., Merle, P., Granito, A., Huang, Y. H., Bodoky, G., et al. (2017). Regorafenib for patients with hepatocellular carcinoma who progressed on sorafenib treatment (RESORCE): A randomised, double-blind, placebo-controlled, phase 3 trial. *Lancet* 389 (10064), 56–66. doi:10.1016/S0140-6736(16)32453-9

Bruix, J., Raoul, J. L., Sherman, M., Mazzaferro, V., Bolondi, L., Craxi, A., et al. (2012). Efficacy and safety of sorafenib in patients with advanced hepatocellular carcinoma: Subanalyses of a phase III trial. *J. Hepatol.* 57 (4), 821–829. doi:10.1016/j.jhep.2012.06.014

Chau, I., Peck-Radosavljevic, M., Borg, C., Malfertheiner, P., Seitz, J. F., Park, J. O., et al. (2017). Ramucirumab as second-line treatment in patients with advanced hepatocellular carcinoma following first-line therapy with sorafenib: Patient-focused outcome results from the randomised phase III REACH study. *Eur. J. Cancer*. 81, 17–25. doi:10.1016/j.ejca.2017.05.001

Chen, B., Garmire, L., Calvisi, D. F., Chua, M. S., Kelley, R. K., and Chen, X. (2020). Harnessing big 'omics' data and AI for drug discovery in hepatocellular carcinoma. *Nat. Rev. Gastroenterol. Hepatol.* 17 (4), 238–251. doi:10.1038/s41575-019-0240-9

Cheng, A. L., Kang, Y. K., Chen, Z., Tsao, C. J., Qin, S., Kim, J. S., et al. (2009). Efficacy and safety of sorafenib in patients in the asia-pacific region with advanced hepatocellular carcinoma: A phase III randomised, double-blind, placebo-controlled trial. *Lancet Oncol.* 10 (1), 25–34. doi:10.1016/S1470-2045(08)70285-7

Collins, F. S., and Varmus, H. (2015). A new initiative on precision medicine. *N. Engl. J. Med.* 372 (9), 793–795. doi:10.1056/NEJMp1500523

Craig, A. J., von Felden, J., Garcia-Lezana, T., Sarcognato, S., and Villanueva, A. (2020). Tumour evolution in hepatocellular carcinoma. *Nat. Rev. Gastroenterol. Hepatol.* 17 (3), 139–152. doi:10.1038/s41575-019-0229-4

Dong, H., Li, Z., Bian, S., Song, G., Song, W., Zhang, M., et al. (2022). Culture of patient-derived multicellular clusters in suspended hydrogel capsules for pre-clinical personalized drug screening. *Bioact. Mater* 18, 164–177. doi:10.1016/j.bioactmat.2022.03.020

EASL Clinical Practice GuidelinesEuropean Association for the Study of the Liver (2018). EASL clinical Practice Guidelines: Management of hepatocellular carcinoma. *J. Hepatol.* 69 (1), 182–236. doi:10.1016/j.jhep.2018.03.019

El-Khoueiry, A. B., Sangro, B., Yau, T., Crocenzi, T. S., Kudo, M., Hsu, C., et al. (2017). Nivolumab in patients with advanced hepatocellular carcinoma (CheckMate 040): An open-label, non-comparative, phase 1/2 dose escalation and expansion trial. *Lancet* 389 (10088), 2492–2502. doi:10.1016/S0140-6736(17)31046-2

Fan, H., Demirci, U., and Chen, P. (2019). Emerging organoid models: Leaping forward in cancer research. *J. Hematol. Oncol.* 12 (1), 142. doi:10.1186/s13045-019-0832-4

Finn Rs, K. M. M. P., Kudo, M., Merle, P., Meyer, T., Qin, S., Ikeda, M., et al. (2022). LBA34 Primary results from the phase III LEAP-002 study: Lenvatinib plus pembrolizumab versus lenvatinib as first-line (1L) therapy for advanced hepatocellular carcinoma (aHCC). *Ann. Oncol.* 33, S1401–S869. doi:10.1016/j.annonc.2022.08.031

Finn, R. S., Merle, P., Granito, A., Huang, Y. H., Bodoky, G., Pracht, M., et al. (2018). Outcomes of sequential treatment with sorafenib followed by regorafenib for HCC: Additional analyses from the phase III RESORCE trial. *J. Hepatol.* 69 (2), 353–358. doi:10.1016/j.jhep.2018.04.010

Finn, R. S., Qin, S., Ikeda, M., Galle, P. R., Ducreux, M., Kim, T. Y., et al. (2020). Atezolizumab plus bevacizumab in unresectable hepatocellular carcinoma. *N. Engl. J. Med.* 382 (20), 1894–1905. doi:10.1056/NEJMoa1915745

Fisher, R., Pusztai, L., and Swanton, C. (2013). Cancer heterogeneity: Implications for targeted therapeutics. *Br. J. Cancer* 108 (3), 479–485. doi:10.1038/bjc.2012.581

Gilabert, M., and Raoul, J. L. (2018). Potential of ramucirumab in treating hepatocellular carcinoma patients with elevated baseline alpha-fetoprotein. *J. Hepatocell. Carcinoma* 5, 91–98. doi:10.2147/JHC.S157413

Gordan, J. D., Kennedy, E. B., Abou-Alfa, G. K., Beg, M. S., Brower, S. T., Gade, T. P., et al. (2020). Systemic therapy for advanced hepatocellular carcinoma: ASCO guideline. *J. Clin. Oncol.* 38 (36), 4317–4345. doi:10.1200/JCO.20.02672

Gu, Y., Forget, A., and Shastri, V. P. (2022). Biobridge: An outlook on translational bioinks for 3D bioprinting. 9 (3), e2103469.doi:10.1002/advs.202103469

Harding, J. J., Nandakumar, S., Armenia, J., Khalil, D. N., Albano, M., Ly, M., et al. (2019). Prospective genotyping of hepatocellular carcinoma: Clinical implications of next-generation sequencing for matching patients to targeted and immune therapies. *Clin. Cancer Res.* 25 (7), 2116–2126. doi:10.1158/1078-0432.CCR-18-2293

He, S., Hu, B., Li, C., Lin, P., Tang, W. G., Sun, Y. F., et al. (2018). PDXliver: A database of liver cancer patient derived xenograft mouse models. *BMC Cancer* 18 (1), 550. doi:10.1186/s12885-018-4459-6

Heinrich, M. A., Bansal, R., Lammers, T., Zhang, Y. S., Michel, S. R., and Prakash, J. (2019). 3D-Bioprinted mini-brain: A glioblastoma model to study cellular interactions and therapeutics. *Adv. Mater.* 31 (14), e1806590. doi:10.1002/adma.201806590

Hernandez-Gea, V., Toffanin, S., Friedman, S. L., and Llovet, J. M. (2013). Role of the microenvironment in the pathogenesis and treatment of hepatocellular carcinoma. *Gastroenterology* 144 (3), 512–527. doi:10.1053/j.gastro.2013.01.002

Hiraoka, A., Kumada, T., Kariyama, K., Takaguchi, K., Atsukawa, M., Itobayashi, E., et al. (2019). Clinical features of lenvatinib for unresectable hepatocellular carcinoma in real-world conditions: Multicenter analysis. *Cancer Med.* 8 (1), 137–146. doi:10.1002/cam4.1909

Hoshida, Y., Nijman, S. M., Kobayashi, M., Chan, J. A., Brunet, J. P., Chiang, D. Y., et al. (2009). Integrative transcriptome analysis reveals common molecular subclasses of human hepatocellular carcinoma. *Cancer Res.* 69 (18), 7385–7392. doi:10.1158/0008-5472.CAN-09-1089

Hu, B., Cheng, J. W., Hu, J. W., Li, H., Ma, X. L., Tang, W. G., et al. (2019). KPNA3 confers sorafenib resistance to advanced hepatocellular carcinoma via TWIST regulated epithelial-mesenchymal transition. *J. Cancer*. 10 (17), 3914–3925. doi:10.7150/jca.31448

Huang, A., Yang, X. R., Chung, W. Y., Dennison, A. R., and Zhou, J. (2020). Targeted therapy for hepatocellular carcinoma. *Signal Transduct. Target Ther.* 5 (1), 146. doi:10.1038/s41392-020-00264-x

Huch, M., Gehart, H., van Boxtel, R., Hamer, K., Blokzijl, F., Verstegen, M. M., et al. (2015). Long-term culture of genome-stable bipotent stem cells from adult human liver. *Cell.* 160 (1-2), 299–312. doi:10.1016/j.cell.2014.11.050

Iavarone, M., Invernizzi, F., Czauderna, C., Sanduzzi-Zamparelli, M., Bhoori, S., Amaddeo, G., et al. (2019). Preliminary experience on safety of regorafenib after sorafenib failure in recurrent hepatocellular carcinoma after liver transplantation. *Am. J. Transpl.* 19 (11), 3176–3184. doi:10.1111/ajt.15551

Ikeda, M., Sung, M. W., Kudo, M., Kobayashi, M., Baron, A. D., Finn, R. S., et al. (2019). A phase ib trial of lenvatinib (LEN) plus pembrolizumab (PEMBRO) in unresectable hepatocellular carcinoma (uHCC): Updated results. *Submitt. Abstr. Gastrointest. TUMOURS, NON-COLORECTAL* 30, V286–V287. doi:10.1093/annonc/mdz247.073

Invrea, F., Rovito, R., Torchiaro, E., Petti, C., Isella, C., and Medico, E. (2020). Patient-derived xenografts (PDXs) as model systems for human cancer. *Curr. Opin. Biotechnol.* 63, 151–156. doi:10.1016/j.copbio.2020.01.003

Jin, H., Shi, Y., Lv, Y., Yuan, S., Ramirez, C., Lieftink, C., et al. (2021). EGFR activation limits the response of liver cancer to lenvatinib. *Nature* 595 (7869), 730–734. doi:10.1038/s41586-021-03741-7

Jung, M., Ghamrawi, S., Du, E. Y., Gooding, J. J., and Kavallaris, M. (2022). Advances in 3D bioprinting for cancer biology and precision medicine: From matrix design to application. *Adv. Healthc. Mater.* 11 (24), e2200690. doi:10.1002/adhm.202200690

Karlovich, C. A., and Williams, P. M. (2019). Clinical applications of next-generation sequencing in precision oncology. *Cancer J.* 25 (4), 264–271. doi:10.1097/PPO.0000000000000385

Knowlton, S., Onal, S., Yu, C. H., Zhao, J. J., and Tasoglu, S. (2015). Bioprinting for cancer research. *Trends Biotechnol.* 33 (9), 504–513. doi:10.1016/j.tibtech.2015.06.007

Kudo, M. (2018). Cabozantinib as a second-line agent in advanced hepatocellular carcinoma. *Liver Cancer* 7 (2), 123–133. doi:10.1159/000488542

Kudo, M., Finn, R. S., Qin, S., Han, K. H., Ikeda, K., Piscaglia, F., et al. (2018). Lenvatinib versus sorafenib in first-line treatment of patients with unresectable hepatocellular carcinoma: A randomised phase 3 non-inferiority trial. *Lancet* 391 (10126), 1163–1173. doi:10.1016/S0140-6736(18)30207-1

Leung, C., Tong, M., Chung, K., Zhou, L., Che, N., Tang, K. H., et al. (2020). Overriding adaptive resistance to sorafenib through combination therapy with src homology 2 domain-containing phosphatase 2 blockade in hepatocellular carcinoma. *Hepatology* 72 (1), 155–168. doi:10.1002/hep.30989

Li, L., Knutsdottir, H., Hui, K., Weiss, M. J., He, J., Philosophe, B., et al. (2019). Human primary liver cancer organoids reveal intratumor and interpatient drug response heterogeneity. *JCI Insight* 4 (2), e121490. doi:10.1172/jci.insight.121490

Lim, J., Kwang, L. G., Ho, N., Toh, C., Too, N., Hooi, L., et al. (2022). Hepatocellular carcinoma organoid co-cultures mimic angiocrine crosstalk to generate inflammatory tumor microenvironment. *Biomaterials* 284, 121527. doi:10.1016/j.biomaterials.2022.121527

Llovet, J. M., Castet, F., Heikenwalder, M., Maini, M. K., Mazzaferro, V., Pinato, D. J., et al. (2022). Immunotherapies for hepatocellular carcinoma. *Nat. Rev. Clin. Oncol.* 19 (3), 151–172. doi:10.1038/s41571-021-00573-2

Llovet, J. M., Kelley, R. K., Villanueva, A., Singal, A. G., Pikarsky, E., Roayaie, S., et al. (2021). Hepatocellular carcinoma. *Nat. Rev. Dis. Prim.* 7 (1), 6. doi:10.1038/s41572-020-00240-3

Llovet, J. M., Montal, R., Sia, D., and Finn, R. S. (2018). Molecular therapies and precision medicine for hepatocellular carcinoma. *Nat. Rev. Clin. Oncol.* 15 (10), 599–616. doi:10.1038/s41571-018-0073-4

Llovet, J. M., Ricci, S., Mazzaferro, V., Hilgard, P., Gane, E., Blanc, J. F., et al. (2008). Sorafenib in advanced hepatocellular carcinoma. *N. Engl. J. Med.* 359 (4), 378–390. doi:10.1056/NEJMoa0708857

Loh, J. J., Li, T. W., Zhou, L., Wong, T. L., Liu, X., Ma, V., et al. (2021). FSTL1 secreted by activated fibroblasts promotes hepatocellular carcinoma metastasis and stemness. *Cancer Res.* 81 (22), 5692–5705. doi:10.1158/0008-5472.CAN-20-4226

Mandrycky, C., Wang, Z., Kim, K., and Kim, D. H. (2016). 3D bioprinting for engineering complex tissues. *Biotechnol. Adv.* 34 (4), 422–434. doi:10.1016/j.biotechadv.2015.12.011

Matai, I., Kaur, G., Seyedsalehi, A., Mcclinton, A., and Laurencin, C. T. (2020). Progress in 3D bioprinting technology for tissue/organ regenerative engineering. *Biomaterials* 226, 119536. doi:10.1016/j.biomaterials.2019.119536

Mcgranahan, N., and Swanton, C. (2017). Clonal heterogeneity and tumor evolution: Past, present, and the future. *Cell.* 168 (4), 613–628. doi:10.1016/j.cell.2017.01.018

Meyer, T., Fox, R., Ma, Y. T., Ross, P. J., James, M. W., Sturgess, R., et al. (2017). Sorafenib in combination with transarterial chemoembolisation in patients with unresectable hepatocellular carcinoma (TACE 2): A randomised placebo-controlled, double-blind, phase 3 trial. *Lancet Gastroenterol. Hepatol.* 2 (8), 565–575. doi:10.1016/S2468-1253(17)30156-5

Montal, R., Andreu-Oller, C., Bassaganyas, L., Esteban-Fabro, R., Moran, S., Montironi, C., et al. (2019). Molecular portrait of high alpha-fetoprotein in hepatocellular carcinoma: Implications for biomarker-driven clinical trials. *Br. J. Cancer* 121 (4), 340–343. doi:10.1038/s41416-019-0513-7

Monteiro, M. V., Zhang, Y. S., Gaspar, V. M., and Mano, J. F. (2022). 3D-bioprinted cancer-on-a-chip: Level-up organotypic *in vitro* models. *Trends Biotechnol.* 40 (4), 432–447. doi:10.1016/j.tibtech.2021.08.007

Murphy, S. V., and Atala, A. (2014). 3D bioprinting of tissues and organs. *Nat. Biotechnol.* 32 (8), 773–785. doi:10.1038/nbt.2958

Nuciforo, S., Fofana, I., Matter, M. S., Blumer, T., Calabrese, D., Boldanova, T., et al. (2018). Organoid models of human liver cancers derived from tumor needle biopsies. *Cell. Rep.* 24 (5), 1363–1376. doi:10.1016/j.celrep.2018.07.001

Ouyang, T., Kan, X., and Zheng, C. (2022). Immune checkpoint inhibitors for advanced hepatocellular carcinoma: Monotherapies and combined therapies. *Front. Oncol.* 12, 898964. doi:10.3389/fonc.2022.898964

Parikh, N. D., Singal, A. G., and Hutton, D. W. (2017). Cost effectiveness of regorafenib as second-line therapy for patients with advanced hepatocellular carcinoma. *Cancer-Am. Cancer Soc.* 123 (19), 3725–3731. doi:10.1002/cncr.30863

Pinyol, R., Montal, R., Bassaganyas, L., Sia, D., Takayama, T., Chau, G. Y., et al. (2019). Molecular predictors of prevention of recurrence in HCC with sorafenib as adjuvant treatment and prognostic factors in the phase 3 STORM trial. *Gut* 68 (6), 1065–1075. doi:10.1136/gutjnl-2018-316408

Qin, B., Kim, K., Goldman, N., Rundle, A. G., Chanumolu, D., Zeinomar, N., et al. (2022). Multilevel factors for adiposity change in a population-based prospective study of black breast cancer survivors. *J. Clin. Oncol.* 40 (20), 2213–2223. doi:10.1200/JCO.21.02973

Qin, S., Ren, Z., Meng, Z., Chen, Z., Chai, X., Xiong, J., et al. (2020). Camrelizumab in patients with previously treated advanced hepatocellular carcinoma: A multicentre, open-label, parallel-group, randomised, phase 2 trial. *Lancet Oncol.* 21 (4), 571–580. doi:10.1016/S1470-2045(20)30011-5

Qu, X. D., Chen, C. S., Wang, J. H., Yan, Z. P., Chen, J. M., Gong, G. Q., et al. (2012). The efficacy of TACE combined sorafenib in advanced stages hepatocellullar carcinoma. *BMC Cancer* 12, 263. doi:10.1186/1471-2407-12-263

Raoul, J. L., Bruix, J., Greten, T. F., Sherman, M., Mazzaferro, V., Hilgard, P., et al. (2012). Relationship between baseline hepatic status and outcome, and effect of sorafenib on liver function: SHARP trial subanalyses. *J. Hepatol.* 56 (5), 1080–1088. doi:10.1016/j.jhep.2011.12.009

Rebouissou, S., and Nault, J. C. (2020). Advances in molecular classification and precision oncology in hepatocellular carcinoma. *J. Hepatol.* 72 (2), 215–229. doi:10.1016/j.jhep.2019.08.017

Ren, Z., Xu, J., Bai, Y., Xu, A., Cang, S., Du, C., et al. (2021). Sintilimab plus a bevacizumab biosimilar (IBI305) versus sorafenib in unresectable hepatocellular carcinoma (ORIENT-32): A randomised, open-label, phase 2-3 study. *Lancet Oncol.* 22 (7), 977–990. doi:10.1016/S1470-2045(21)00252-7

Rygaard, J., and Povlsen, C. O. (1969). Heterotransplantation of a human malignant tumour to "Nude" mice. *Acta Pathol. Microbiol. Scand.* 77 (4), 758–760. doi:10.1111/j.1699-0463.1969.tb04520.x

Sato, T., Stange, D. E., Ferrante, M., Vries, R. G., Van Es, J. H., Van den Brink, S., et al. (2011). Long-term expansion of epithelial organoids from human colon, adenoma, adenocarcinoma, and Barrett's epithelium. *Gastroenterology* 141 (5), 1762–1772. doi:10.1053/j.gastro.2011.07.050

Sbirkov, Y., Molander, D., Milet, C., Bodurov, I., Atanasov, B., Penkov, R., et al. (2021). A colorectal cancer 3D bioprinting workflow as a platform for disease modeling and chemotherapeutic screening. *Front. Bioeng. Biotechnol.* 9, 755563. doi:10.3389/fbioe.2021.755563

Schulze, K., Imbeaud, S., Letouze, E., Alexandrov, L. B., Calderaro, J., Rebouissou, S., et al. (2015). Exome sequencing of hepatocellular carcinomas identifies new mutational signatures and potential therapeutic targets. *Nat. Genet.* 47 (5), 505–511. doi:10.1038/ng.3252

Shukla, P., Yeleswarapu, S., Heinrich, M. A., Prakash, J., and Pati, F. (2022). Mimicking tumor microenvironment by 3D bioprinting: 3D cancer modeling. *Biofabrication* 14 (3), 032002. doi:10.1088/1758-5090/ac6d11

Soto-Perez-De-Celis, E., Aguiar, P. N., Cordon, M. L., Chavarri-Guerra, Y., and Lopes, G. L. (2019). Cost-effectiveness of cabozantinib in the second-line treatment of advanced hepatocellular carcinoma. *J. Natl. Compr. Canc Netw.* 17 (6), 669–675. doi:10.6004/jnccn.2018.7275

Sun, F. X., Tang, Z. Y., Lui, K. D., Ye, S. L., Xue, Q., Gao, D. M., et al. (1996). Establishment of a metastatic model of human hepatocellular carcinoma in nude mice via orthotopic implantation of histologically intact tissues. *Int. J. Cancer*. 66 (2), 239–243. doi:10.1002/(SICI)1097-0215(19960410)66:2<239:AID-IJC17>3.0.CO;2-7

Sun, L., Yang, H., Wang, Y., Zhang, X., Jin, B., Xie, F., et al. (2020). Application of a 3D bioprinted hepatocellular carcinoma cell model in antitumor drug research. *Front. Oncol.* 10, 878. doi:10.3389/fonc.2020.00878

Swaminathan, S., Hamid, Q., Sun, W., and Clyne, A. M. (2019). Bioprinting of 3D breast epithelial spheroids for human cancer models. *Biofabrication* 11 (2), 025003. doi:10.1088/1758-5090/aafc49

Syed, Y. Y. (2020). Ramucirumab: A review in hepatocellular carcinoma. *Drugs* 80 (3), 315–322. doi:10.1007/s40265-020-01263-6

Takebe, T., Sekine, K., Enomura, M., Koike, H., Kimura, M., Ogaeri, T., et al. (2013). Vascularized and functional human liver from an iPSC-derived organ bud transplant. *Nature* 499 (7459), 481–484. doi:10.1038/nature12271

Tang, W., Chen, Z., Zhang, W., Cheng, Y., Zhang, B., Wu, F., et al. (2020). The mechanisms of sorafenib resistance in hepatocellular carcinoma: Theoretical basis and therapeutic aspects. *Signal Transduct. Target Ther.* 5 (1), 87. doi:10.1038/s41392-020-0187-x

Teufel, M., Seidel, H., Kochert, K., Meinhardt, G., Finn, R. S., Llovet, J. M., et al. (2019). Biomarkers associated with response to regorafenib in patients with hepatocellular carcinoma. *Gastroenterology* 156 (6), 1731–1741. doi:10.1053/j.gastro.2019.01.261

Tharehalli, U., Svinarenko, M., and Lechel, A. (2019). Remodelling and improvements in organoid technology to study liver carcinogenesis in a dish. *Stem Cells Int.* 2019, 3831213. doi:10.1155/2019/3831213

Tuveson, D., and Clevers, H. (2019). Cancer modeling meets human organoid technology. *Science* 364 (6444), 952–955. doi:10.1126/science.aaw6985

Villanueva, A. (2019). Hepatocellular carcinoma. *N. Engl. J. Med.* 380 (15), 1450–1462. doi:10.1056/NEJMra1713263

Vogel, A., Meyer, T., Sapisochin, G., Salem, R., and Saborowski, A. (2022). Hepatocellular carcinoma. *Lancet* 400 (10360), 1345–1362. doi:10.1016/S0140-6736(22)01200-4

Wang, X., Zhang, X., Dai, X., Wang, X., Li, X., Diao, J., et al. (2018). Tumor-like lung cancer model based on 3D bioprinting. *3 Biotech.* 8 (12), 501. doi:10.1007/s13205-018-1519-1

Wang, S., Wang, Y., Xun, X., Zhang, C., Xiang, X., Cheng, Q., et al. (2020). Hedgehog signaling promotes sorafenib resistance in hepatocellular carcinoma patient-derived organoids. *J. Exp. Clin. Cancer Res.* 39 (1), 22. doi:10.1186/s13046-020-1523-2

Weis, S. M., and Cheresh, D. A. (2011). Tumor angiogenesis: Molecular pathways and therapeutic targets. *Nat. Med.* 17 (11), 1359–1370. doi:10.1038/nm.2537

Xie, F., Sun, L., Pang, Y., Xu, G., Jin, B., Xu, H., et al. (2021). Three-dimensional bio-printing of primary human hepatocellular carcinoma for personalized medicine. *Biomaterials* 265, 120416. doi:10.1016/j.biomaterials.2020.120416

Xu, J., Shen, J., Gu, S., Zhang, Y., Wu, L., Wu, J., et al. (2021). Camrelizumab in combination with apatinib in patients with advanced hepatocellular carcinoma (RESCUE): A nonrandomized, open-label, phase II trial. *Clin. Cancer Res.* 27 (4), 1003–1011. doi:10.1158/1078-0432.CCR-20-2571

Yoo, C., Park, J. W., Kim, Y. J., Kim, D. Y., Yu, S. J., Lim, T. S., et al. (2019). Multicenter retrospective analysis of the safety and efficacy of regorafenib after progression on sorafenib in Korean patients with hepatocellular carcinoma. *Invest New Drugs* 37 (3), 567–572. doi:10.1007/s10637-018-0707-5

Zucman-Rossi, J., Villanueva, A., Nault, J. C., and Llovet, J. M. (2015). Genetic landscape and biomarkers of hepatocellular carcinoma. *Gastroenterology* 149 (5), 1226–1239. doi:10.1053/j.gastro.2015.05.061

Zeng, Q., Klein, C., Caruso, S., Maille, P., Laleh, N. G., Sommacale, D., et al. (2022). Artificial intelligence predicts immune and inflammatory gene signatures directly from hepatocellular carcinoma histology. *J. Hepatol.* 77 (1), 116–127. doi:10.1016/j.jhep.2022.01.018

Zhao, Y., Shuen, T., Toh, T. B., Chan, X. Y., Liu, M., Tan, S. Y., et al. (2018). Development of a new patient-derived xenograft humanised mouse model to study human-specific tumour microenvironment and immunotherapy. *Gut* 67 (10), 1845–1854. doi:10.1136/gutjnl-2017-315201

Zhao, Y., Zhu, X., Wang, H., Dong, D., Gao, S., Zhu, X., et al. (2019). Safety and efficacy of transcatheter arterial chemoembolization plus radiotherapy combined with sorafenib in hepatocellular carcinoma showing macrovascular invasion. *Front. Oncol.* 9, 1065. doi:10.3389/fonc.2019.01065

Zhou, X., Zhu, W., Nowicki, M., Miao, S., Cui, H., Holmes, B., et al. (2016). 3D bioprinting a cell-laden bone matrix for breast cancer metastasis study. *ACS Appl. Mater Interfaces* 8 (44), 30017–30026. doi:10.1021/acsami.6b10673

Zhu, A. X., Baron, A. D., Malfertheiner, P., Kudo, M., Kawazoe, S., Pezet, D., et al. (2017). Ramucirumab as second-line treatment in patients with advanced hepatocellular carcinoma: Analysis of REACH trial results by child-pugh score. *JAMA Oncol.* 3 (2), 235–243. doi:10.1001/jamaoncol.2016.4115

Zhu, A. X., Finn, R. S., Kang, Y. K., Yen, C. J., Galle, P. R., Llovet, J. M., et al. (2021). Serum alpha-fetoprotein and clinical outcomes in patients with advanced hepatocellular carcinoma treated with ramucirumab. *Br. J. Cancer* 124 (8), 1388–1397. doi:10.1038/s41416-021-01260-w

Zhu, A. X., Kang, Y. K., Yen, C. J., Finn, R. S., Galle, P. R., Llovet, J. M., et al. (2019). Ramucirumab after sorafenib in patients with advanced hepatocellular carcinoma and increased α-fetoprotein concentrations (REACH-2): A randomised, double-blind, placebo-controlled, phase 3 trial. *Lancet Oncol.* 20 (2), 282–296. doi:10.1016/S1470-2045(18)30937-9

Zhu, A. X., Park, J. O., Ryoo, B. Y., Yen, C. J., Poon, R., Pastorelli, D., et al. (2015). Ramucirumab versus placebo as second-line treatment in patients with advanced hepatocellular carcinoma following first-line therapy with sorafenib (REACH): A randomised, double-blind, multicentre, phase 3 trial. *Lancet Oncol.* 16 (7), 859–870. doi:10.1016/S1470-2045(15)00050-9

frontiers | Frontiers in Pharmacology

TYPE Original Research
PUBLISHED 14 June 2023
DOI 10.3389/fphar.2023.1159286



OPEN ACCESS

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RECEIVED 05 February 2023
ACCEPTED 05 June 2023
PUBLISHED 14 June 2023

CITATION
Wang C-C, Yu C-Y, Zhang J, Wang R and Kong X-S (2023), A bibliometric study on the utilization of lenvatinib in hepatocellular carcinoma (2014–2022). *Front. Pharmacol.* 14:1159286. doi: 10.3389/fphar.2023.1159286



# A bibliometric study on the utilization of lenvatinib in hepatocellular carcinoma (2014–2022)

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**Background:** The REFLECT phase-III trial has demonstrated the efficacy of lenvatinib in improving the overall survival of advanced hepatocellular carcinoma (HCC) patients, comparable to sorafenib. The rapidly evolving landscape of hepatocellular carcinoma therapy presents new avenues for lenvatinib. This study aims to provide a scientometric analysis of publications and predict research hotspots in this field.

**Methods:** Relevant publications were sourced from the Web of Science Core Collection (WoSCC) database up until November 2022. The bibliometrix tool in R was employed for scientometric analysis and visualization.

**Results:** A total of 879 publications from 2014 to 2022 were obtained from WoSCC that met the established criteria. These studies involved 4,675 researchers from 40 countries, with an average annual growth rate of 102.5%. The highest number of publications was from Japan, followed by China, Italy, and the United States. The largest proportion of studies, 14.0% ($n$ = 123), was contributed by FUDAN UNIV. The studies were published in 274 journals, with CANCERS ($n$ = 53) being the top journal, followed by FRONTIERS IN ONCOLOGY ($n$ = 51) and HEPATOLOGY RESEARCH ($n$ = 36). The top ten journals accounted for 31.5% of the 879 studies. The most prolific authors were Kudo M ($n$ = 51), Hiraoka A ($n$ = 43), and Tsuji K ($n$ = 38). A total of 1,333 keywords were analyzed, with the present research hotspots being "immune checkpoint inhibitors," "prognosis," and "pd-1." Co-occurrence clustering analysis revealed the top keywords, authors, publications, and journals. Strong collaboration was identified in the field.

**Conclusion:** This scientometric and visual analysis provides a comprehensive summary of the published articles on lenvatinib in HCC during 2014–2022, highlighting the research hotspots, knowledge domain, and frontiers. The results can provide insights into future research directions in this field.

KEYWORDS

**bibliometric, lenvatinib, hepatocellular carcinoma, prognosis, immunotherapy**

# 1 Introduction

Hepatocellular carcinoma (HCC) is a prevalent solid tumor that is a major contributor to cancer-related mortality globally (Zhou et al., 2020). Unfortunately, over half of HCC cases are diagnosed at moderate-to-advanced stages (Marrero et al., 2018), which results in poor patient prognoses due to heavy tumor burdens, liver function impairment, and health deterioration, leading to limited treatment options.

Sorafenib, a first-line systemic treatment, was the only therapeutic agent available from 2007 to 2017 and was proven to improve overall survival (OS) compared to placebo in randomized controlled trials (RCTs) (Llovet et al., 2008; Bruix et al., 2017). A phase-III REFLECT trial (Kudo et al., 2018) reported that lenvatinib was as effective as sorafenib in improving OS (13.6 vs. 12.3 months) and superior in improving objective response rate (ORR, 41% vs. 12%) and progression-free survival (PFS, 7.3 vs. 3.6 months) in advanced HCC cases. As a result, international guidelines now recommend lenvatinib as the first-line treatment for advanced HCC.

In recent years, immune checkpoint inhibitors (ICIs) have shown favorable outcomes in HCC treatment. The IMbrave150 study (Finn et al., 2020a) used a combination of atezolizumab and bevacizumab as first-line therapy for advanced HCC and reported improved outcomes such as OS, PFS, disease control rate (DCR), and ORR compared to sorafenib monotherapy. However, the combination of atezolizumab and bevacizumab was not cost-effective prior to a substantial price reduction (Zhang et al., 2021). Lenvatinib is also recommended by international guidelines as a first-line treatment for HCC, but the place of lenvatinib monotherapy or in combination with ICIs in second-line treatment following ICIs has yet to be consistently determined due to insufficient clinical trial data.

Despite the growing number of studies on lenvatinib in HCC, no specific scientometric analysis of its knowledge structure has been conducted. In this study, we conducted the first scientometric analysis of articles on lenvatinib application in HCC, utilizing literature metrological features to evaluate our research outcomes, influence, and cooperation, identify hotspots, and discuss future trends in this field.

# 2 Materials and methods

## 2.1 Data extraction

In the present work, we have employed a rigorous data extraction process to obtain the relevant information on lenvatinib in the context of hepatocellular carcinoma. The Web of Science Core Collection database, a well-respected and high-quality database, was comprehensively searched using the search terms of "lenvatinib" and "hepatocellular carcinoma" up to November 2022. Only articles and reviews published in English that met our eligibility criteria were selected for further analysis, with the "full record and cited references" being the output. Two independent reviewers were involved in this process to ensure the accuracy of the scientometric analysis. The collected data included title, authors, institution, country/region, journal, abstract, keywords, and references, while data papers, book chapters, proceedings papers or meeting abstracts, editorials, duplicates, or unpublished articles were excluded. Any

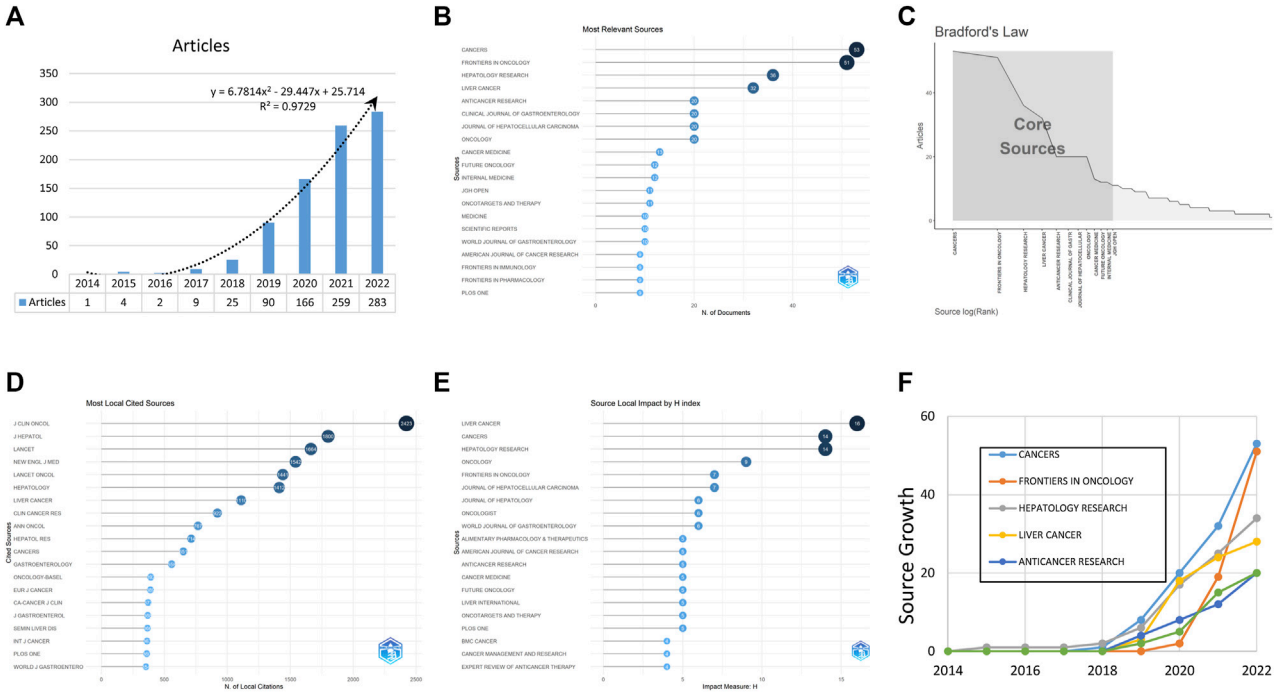


FIGURE 1
The fundamental data of publications and journals, including the annual scientific study production **(A)**, the most relevant journals **(B)**, the core journals **(C)**, the most locally cited sources **(D)**, the journal impact adjusted by the H index **(E)**, and the journal production over time **(F)**.

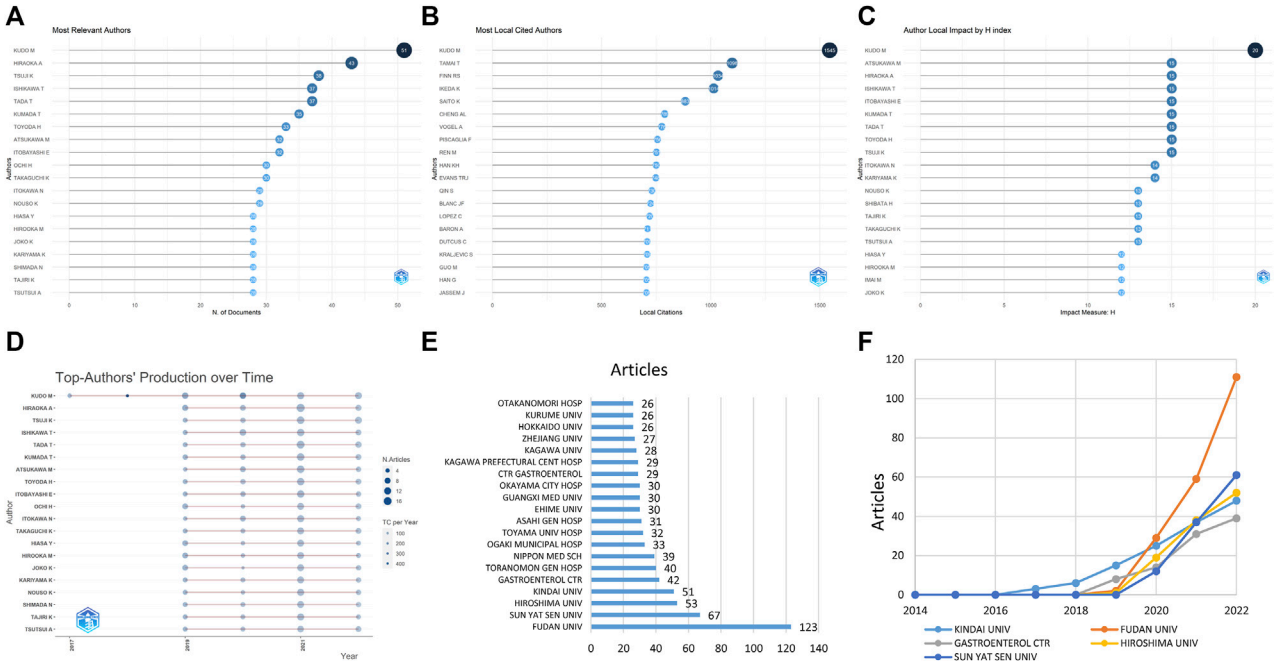


FIGURE 2
The distributions of authors and institutions. It depicts the most publications by authors **(A)**, the most locally cited authors **(B)**, the author impact adjusted by the H index **(C)**, and the authors with the highest publication number over time **(D)**. The line stands for the timeline of an author from 2017 to 2020 and the color intensity and bubble size positively correspond to total annual citations and document number, respectively. The figure also displays the most publications by institutions **(E)** and the production of institutions over time **(F)**.

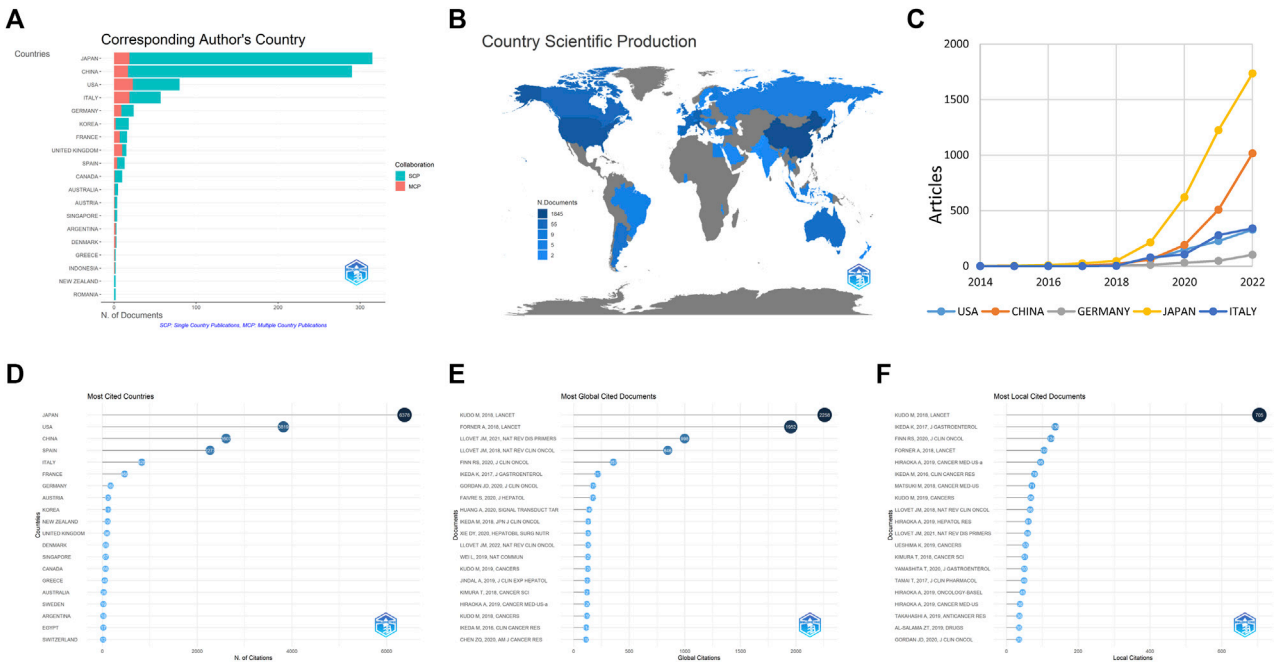


FIGURE 3
Contributions of different countries, including the top twenty countries with the most articles **(A)**, the production of scientific studies in global countries **(B)**, the top five countries with the greatest number of publications over time **(C)**, the top twenty countries with the greatest citations **(D)**, the top twenty globally cited documents **(E)**, and the top twenty locally cited documents **(F)**. The SCP and MCP refer to single country publications and multiple country publications, respectively.

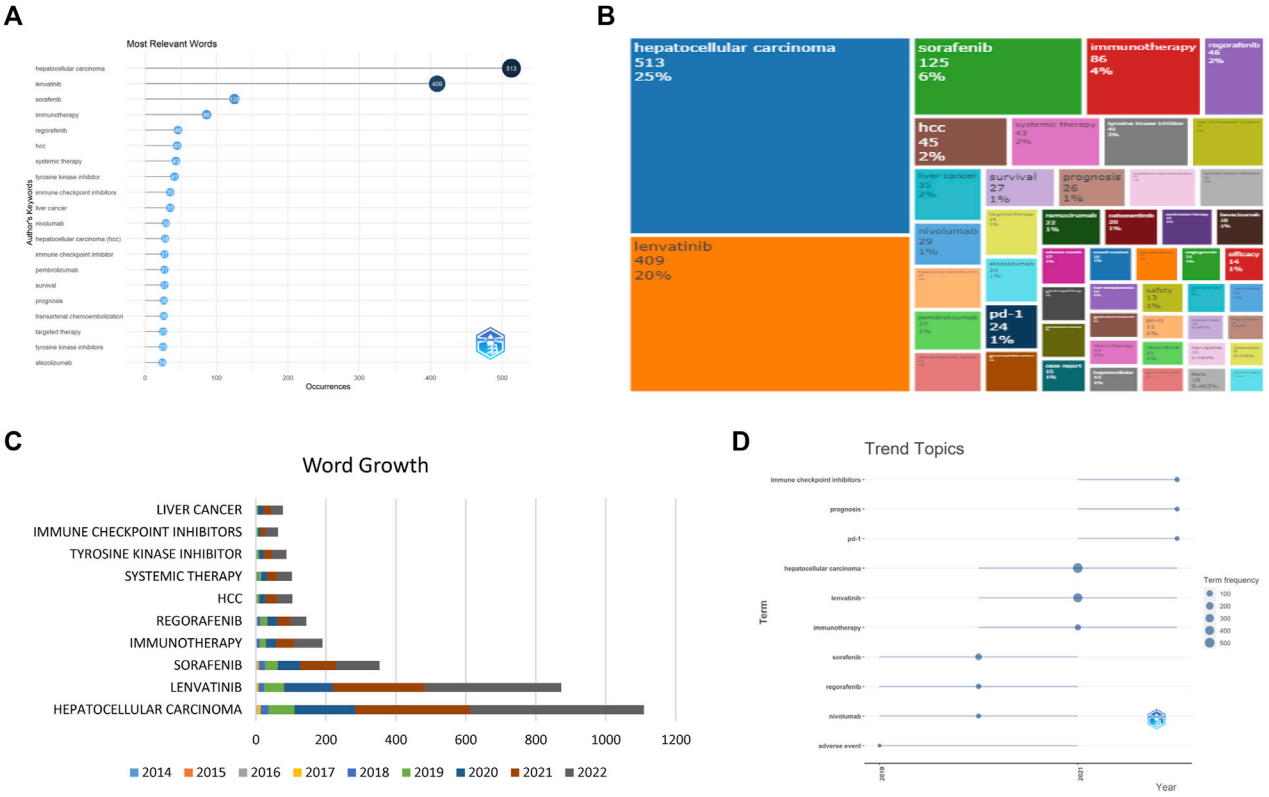


FIGURE 4
The keyword revolution, including the top keywords with high frequency **(A,B)**, the top ten keywords with the highest accumulation **(C)**, and the trends of the top ten keywords **(D)**.

disagreements were resolved through negotiation or consultation with a senior physician.

## 2.2 Statistical methods

For the purpose of scientometric analysis and visualization, we have utilized the R-language package Bibliometrix (Aria and Cuccurullo, 2017). Bibliometrix is a comprehensive and flexible package that provides automatic algorithms and machine intelligence to collect and examine the data. It was used in this work to obtain information on basic data, cited references, trend topics, and landmark literature in the field of lenvatinib in hepatocellular carcinoma, as well as to analyze countries, journals, author productivity, and institutions.

# 3 Results

## 3.1 General features of published study

In the analysis of published studies on the topic, a total of 879 studies meeting the eligibility criteria were collected in WoSCC. This involved 4,675 authors globally, with an average annual increase rate of 102.5%. The number of published studies increased over time, with a particularly fast increase trend after 2017, accounting for 94.7% of all published studies (Figure 1A).

## 3.2 Analysis of journals

The studies were published in 274 journals, with the highest number in CANCERS ($n$ = 53), followed by FRONTIERS IN ONCOLOGY ($n$ = 51) and HEPATOLOGY RESEARCH ($n$ = 36). The top ten most productive journals accounted for 31.5% of the 879 published studies (Figure 1B) and were identified using Bradford's law (Figure 1C). Bibliometrix was used to analyze frequently cited sources, with the top three journals being J CLIN ONCOL, J HEPATOL and LANCET (Figure 1D). After adjustment by the H index, the leading journals were LIVER CANCER, CANCERS, and HEPATOLOGY RESEARCH (Figure 1E). The cumulative production of the top five journals over time is displayed in Figure 1F.

## 3.3 Sources of author and institution

Regarding the authors and institutions, there were 5 authors who published over 35 papers, with Kudo M ($n$ = 51) being the most productive, followed by Hiraoka A ($n$ = 43) and Tsuji K ($n$ = 38)

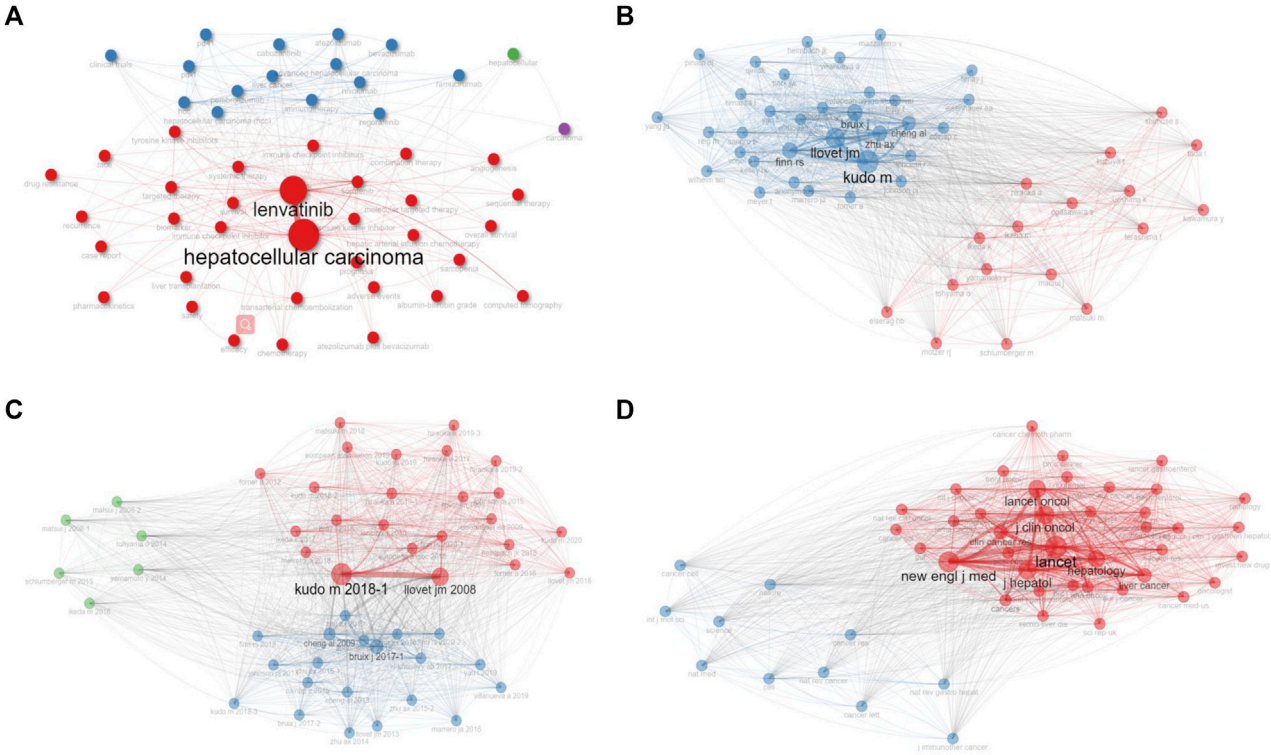


FIGURE 5
The clustering analysis of keywords **(A)**, authors **(B)**, documents **(C)**, and journals **(D)**, with node size and edge size positively correlated with item occurrence and item co-occurrences, respectively. Nodes with the same colors belong to the same cluster.

(Figure 2A). Kudo M, Tamai T, Finn RS, and Ikeda K were the most frequently cited authors, with more than 1,000 citations (Figure 2B). After adjustment by the H index, Kudo M remained the top author (Figure 2C). The authors' production over time is shown in Figure 2D, with Kudo M having the longest timeline from 2017 to 2022.

The top 20 institutions producing the most publications are illustrated in Figure 2E, with the top two being from China (FUDAN UNIV and SUN YAT SEN UNIV). FUDAN UNIV was the most productive institution, publishing 123 studies, accounting for 14.0% of the 879 studies (Figure 2F). Although FUDAN UNIV and SUN YAT SEN UNIV in China only started publishing studies on this topic in 2020, they have continued to increase their publication numbers.

## 3.4 Analysis of countries and most cited publications

According to the country of corresponding authors, Japan published the highest number of studies, followed by China and the United States (Figure 3A). The top five countries were in three continents, with two in Asia (Japan and China), one in North America (the United States), and two in Europe (Germany and Italy). Japan had a clear advantage in the number of published studies and an increase in relative number compared to the rest of the countries (Figures 3B, C). Among the top 20 countries with the most publications, Japan also had the highest mean citation rate (Figure 3D). The study by Kudo M published in LANCET in 2018 ranked first in both global (Figure 3E) and local (Figure 3F) citations, indicating its high quality.

## 3.5 Investigation of keyword

In our examination of the 879 studies, a total of 1,333 keywords were collected. As depicted in Figures 4A, B, the most frequently used keywords were HCC, lenvatinib, sorafenib, immunotherapy, and regorafenib, demonstrating the significance of these topics in the research. Furthermore, our analysis of the keyword occurrence trend (Figures 4C, D) revealed that the most recent keywords of interest are "ICI", "prognosis", and "PD-1", offering insight into potential future research directions.

## 3.6 Analysis of cluster and collaboration network

Our examination also included a cluster and collaboration network analysis, as represented in Figure 5. These figures display mathematical structures modeling the relationships between the keywords, authors, documents, and journals, where node size and edge size reflect item occurrence and item co-occurrences, respectively. The results indicate that

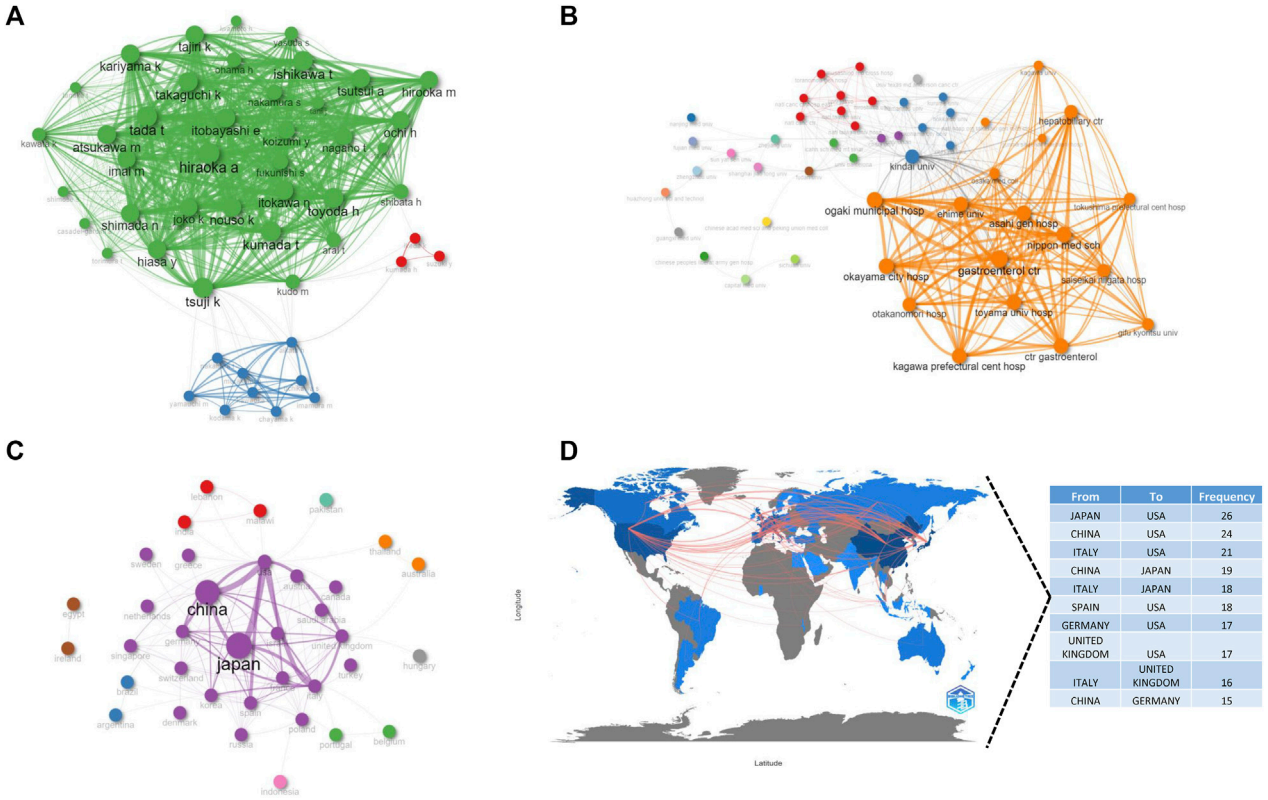


FIGURE 6
The collaboration network, including the authors **(A)**, institutions **(B)**, countries **(C)**, and the cooperation of countries in contributing to publications **(D)**. Node size and edge size positively correspond to item occurrence and item co-occurrences, respectively, and nodes with the same colors belong to the same cluster.

Lenvatinib, Kudo M, Kudo M's 2018 document, and LANCET hold the highest centrality in their respective clusters.

In addition, we visually depicted international collaboration relationships among authors (Figure 6A), institutions (Figure 6B), and countries (Figures 6C, D). These findings suggest tight cooperation among authors and institutions from Japan, as well as close connections among the top four countries with the most publications, indicating that inter-country collaboration plays a crucial role in research outcome achievement.

## 4 Discussion

In this study, a comprehensive scientometric and visualized analysis was conducted on studies regarding lenvatinib treatment in HCC between 2014 and 2022. Adopting a bibliometric approach provided a more in-depth understanding of research trends and hotspots, and was more objective and thorough compared to traditional methods. A total of 879 published studies were analyzed in this work. The number of publications rapidly increased since 2017, and the most productive countries, institutions, and authors were also identified. According to the keyword distribution analysis, current research hotspots include "ICI," "prognosis," and "PD-1." This analysis provides valuable insight into the evolution of the field, contributing to its development.

Studies on the application of lenvatinib in HCC first appeared in 2014 (Kumar and Huang, 2014), attracting significant attention from researchers. In 2017, the landmark study conducted by Japanese researcher Kudo M and published in LANCET (the REFLECT trial) demonstrated that lenvatinib was as effective as sorafenib in improving OS. The REFLECT trial established critical eligibility criteria, including the absence of surgical indications, moderate-to-advanced stage based on the Barcelona Clinic Liver Cancer system, Child-Pugh class A, and the absence of prior systemic treatment. The results showed that lenvatinib was as effective as sorafenib in improving OS (13.6 vs. 12.3 months; hazard ratio (HR) = 0.92) and had a higher ORR (40.6% and 18.8% by mRECIST and RECIST ver.1.1, separately), reduced time-to-progression (median, 7.4 months), and improved PFS (median, 7.3 months). Following the REFLECT trial, lenvatinib was recommended for moderate-to-advanced stage HCC patients who had progressive disease following transarterial chemoembolization by the American Association for the Study of Liver Diseases in their 2018 guidelines. Additionally, it was also recommended for advanced stage HCC patients and HCC patients with progressive disease or not suitable for locoregional treatments among Child-Pugh class A patients or those with good performance

status by the European Association for the Study of the Liver (European Association for the Study of the Liver, 2018). The study captured the critical research by Kudo M, a highly active and renowned author known for his contributions to the exploration of lenvatinib and its underlying mechanisms.

ICIs represent a groundbreaking strategy in the ongoing revolution in cancer treatment. As more studies shed light on the mechanisms by which tumor cells evade immune attack, great attention has been directed towards ICIs (Rizzo et al., 2021). In 2021, the combination of atezolizumab and bevacizumab was recommended as a first-line therapy for unresectable HCC cases due to its superior survival rate compared to sorafenib (Benson et al., 2021). However, as a recent multi-center study suggests, lenvatinib treatment may offer more significant survival benefits when compared to the atezolizumab and bevacizumab combination (Rimini et al., 2022). On the other hand, a phase-III RCT found that anti-PD-1 treatment did not improve survival significantly in advanced HCC cases, as drug resistance was observed in some cases (Finn et al., 2020b). A recent article (Wei et al., 2022) explored the resistance mechanism and suggested a critical role for the PKCa/ZFP64/CSF1 pathway in facilitating immune evasion. Notably, lenvatinib was found to downregulate PKC levels and suppress the PKCa/ZFP64/CSF1 pathway, thus overcoming resistance to anti-PD-1 treatment in HCC, unlike sorafenib. Moreover, a real-world study found that lenvatinib combined with sintilimab produced better long-term results than lenvatinib monotherapy (Zhao et al., 2022). Consequently, lenvatinib holds promise as a monotherapy and in combination with ICIs as a novel treatment option for unresectable HCC cases in clinical practice. It is important to note that combination treatment may result in ICI-related adverse events, and personalized dosing may help mitigate these events while maximizing patient outcomes (Zhao et al., 2022).

Our study provides an overview of the current research landscape and identifies key trends and future prospects in the field of lenvatinib treatment in HCC. Preclinical and clinical research have shown that lenvatinib in combination with ICIs is effective and safe in treating cancers. For example, lenvatinib combined with pembrolizumab has been approved as a second-line treatment for advanced endometrial cancer that has failed systemic treatment (Makker et al., 2020). A phase-1b trial of lenvatinib and pembrolizumab in advanced HCC cases showed favorable antitumor effects with a median OS of 22 months and an acceptable toxicity profile (Finn et al., 2020c). Additionally, the ORR reached 46.0% under mRECIST criteria, with 11 cases achieving complete response and median response duration and PFS of 8.6 and 9.3 months, respectively. In intermediate HCC patients eligible for locoregional treatment, a phase-III trial (LEAP-012) was conducted comparing lenvatinib and pembrolizumab to placebo and TACE (Llovet et al., 2022). The latest published studies shed light on the clinical value of lenvatinib in HCC and provide insight into the current options for systemic treatment (Li et al., 2022; Jiang et al., 2023; Leowattana et al., 2023; Su et al., 2023; Xie et al., 2023; Yang et al., 2023).

Japan, China, Itlay and the United States were the most productive countries, and close collaborations were observed between countries and institutions. However, collaborations between United States and other countries were found to be stronger than those between countries outside United States, implying that international collaborations should be strengthened.

It must be noted that certain limitations exist in our analysis. Firstly, the studies included in this analysis were sourced from only the WoSCC database, potentially causing a biased representation of the data. Secondly, with the recent increase in the number of studies published on the topic, citation counts may not accurately reflect the more recent advancements. Furthermore, alternative bibliometric software utilizing diverse algorithms are available, although the R software employed in our analysis proves to be a powerful tool, it is not without limitations.

In conclusion, this scientometric and visual analysis delves into the data surrounding the application of lenvatinib in HCC, covering the period from 2014 to 2022. Our results offer valuable insights into the treatment of HCC with lenvatinib, serving as a guiding light for future research and the discovery of innovative treatment regimens.

## Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding authors.

## Author contributions

X-SK and RW designed this study. C-CW, C-YY, and JZ performed the search and collected data. C-CW, C-YY, and JZ performed analysis. All authors contributed to the article and approved the submitted version.

## Funding

This study was supported by WUJIEPING Medical Foundation (320.6750.2022-22-47).

## Acknowledgments

We thank the global researchers who contributed to this topic.

## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

## Publisher's note

# References

Aria, M., and Cuccurullo, C. (2017). bibliometrix: An R-tool for comprehensive science mapping analysis. *J. Informetr.* 11 (4), 959–975. PubMed PMID: WOS:000418020600003. doi:10.1016/j.joi.2017.08.007

Benson, A. B., D'Angelica, M. I., Abbott, D. E., Anaya, D. A., Anders, R., Are, C., et al. (2021). Hepatobiliary cancers, version 2.2021, NCCN clinical practice guidelines in oncology. *J. Natl. Compr. Cancer Netw.* 19 (5), 541–565. PubMed PMID: 34030131. doi:10.6004/jnccn.2021.0022

Bruix, J., Cheng, A. L., Meinhardt, G., Nakajima, K., De Sanctis, Y., and Llovet, J. (2017). Prognostic factors and predictors of sorafenib benefit in patients with hepatocellular carcinoma: Analysis of two phase III studies. *J. hepatology* 67 (5), 999–1008. PubMed PMID: 28687477. doi:10.1016/j.jhep.2017.06.026

European Association for the Study of the Liver (2018). EASL clinical practice guidelines: Management of hepatocellular carcinoma. *J. hepatology* 69 (1), 182–236. PubMed PMID: 29628281. doi:10.1016/j.jhep.2018.03.019

Finn, R. S., Ikeda, M., Zhu, A. X., Sung, M. W., Baron, A. D., Kudo, M., et al. (2020c). Phase ib study of lenvatinib plus pembrolizumab in patients with unresectable hepatocellular carcinoma. *J. Clin. Oncol.* 38 (26), 2960–2970. PubMed PMID: 32716739; PubMed Central PMCID: PMCPMC7479760. doi:10.1200/JCO.20.00808

Finn, R. S., Qin, S., Ikeda, M., Galle, P. R., Ducreux, M., Kim, T. Y., et al. (2020a). Atezolizumab plus bevacizumab in unresectable hepatocellular carcinoma. *N. Engl. J. Med.* 382 (20), 1894–1905. PubMed PMID: 32402160. doi:10.1056/NEJMoa1915745

Finn, R. S., Ryoo, B. Y., Merle, P., Kudo, M., Bouattour, M., Lim, H. Y., et al. (2020b). Pembrolizumab as second-line therapy in patients with advanced hepatocellular carcinoma in KEYNOTE-240: A randomized, double-blind, phase III trial. *J. Clin. Oncol.* 38 (3), 193–202. PubMed PMID: 31790344. doi:10.1200/JCO.19.01307

Jiang, L., Li, L., Liu, Y., Lu, L., Zhan, M., Yuan, S., et al. (2023). Drug resistance mechanism of kinase inhibitors in the treatment of hepatocellular carcinoma. *Front. Pharmacol.* 14, 1097277. PubMed PMID: 36891274; PubMed Central PMCID: PMCPMC9987615. doi:10.3389/fphar.2023.1097277

Kudo, M., Finn, R. S., Qin, S., Han, K. H., Ikeda, K., Piscaglia, F., et al. (2018). Lenvatinib versus sorafenib in first-line treatment of patients with unresectable hepatocellular carcinoma: A randomised phase 3 non-inferiority trial. *Lancet* 391 (10126), 1163–1173. PubMed PMID: 29433850. doi:10.1016/S0140-6736(18)30207-1

Kumar, A. K. L., and Huang, C. H. (2014). Lenvatinib mesylate multikinase inhibitor oncolytic. *Drug Future* 39 (2), 113–122. PubMed PMID: WOS:000332213500002. doi:10.1358/dof.2014.39.2.2095258

Leowattana, W., Leowattana, T., and Leowattana, P. (2023). Systemic treatment for unresectable hepatocellular carcinoma. *World J. gastroenterology* 29 (10), 1551–1568. PubMed PMID: 36970588; PubMed Central PMCID: PMCPMC10037251. doi:10.3748/wjg.v29.i10.1551

Li, X., Wang, J., Ding, X., Xu, Y., Yu, M., Wu, H., et al. (2022). Clinical study of lenvatinib in the treatment of hepatitis virus-related hepatocellular carcinoma and antiviral therapy. *Front. Pharmacol.* 13, 1032881. PubMed PMID: 36703739; PubMed Central PMCID: PMCPMC9871375. doi:10.3389/fphar.2022.1032881

Llovet, J. M., Ricci, S., Mazzaferro, V., Hilgard, P., Gane, E., Blanc, J. F., et al. (2008). Sorafenib in advanced hepatocellular carcinoma. *N. Engl. J. Med.* 359 (4), 378–390. PubMed PMID: 18650514. doi:10.1056/NEJMoa0708857

Llovet, J. M., Vogel, A., Madoff, D. C., Finn, R. S., Ogasawara, S., Ren, Z., et al. (2022). Randomized phase 3 LEAP-012 study: Transarterial chemoembolization with or without lenvatinib plus pembrolizumab for intermediate-stage hepatocellular carcinoma not amenable to curative treatment. *Cardiovasc. interventional radiology* 45 (4), 405–412. PubMed PMID: 35119481; PubMed Central PMCID: PMCPMC8940827. doi:10.1007/s00270-021-03031-9

Makker, V., Taylor, M. H., Aghajanian, C., Oaknin, A., Mier, J., Cohn, A. L., et al. (2020). Lenvatinib plus pembrolizumab in patients with advanced endometrial cancer. *J. Clin. Oncol.* 38 (26), 2981–2992. PubMed PMID: 32167863; PubMed Central PMCID: PMCPMC7479759. doi:10.1200/JCO.19.02627

Marrero, J. A., Kulik, L. M., Sirlin, C. B., Zhu, A. X., Finn, R. S., Abecassis, M. M., et al. (2018). Diagnosis, staging, and management of hepatocellular carcinoma: 2018 practice guidance by the American association for the study of liver diseases. *Hepatology* 68 (2), 723–750. PubMed PMID: 29624699. doi:10.1002/hep.29913

Rimini, M., Rimassa, L., Ueshima, K., Burgio, V., Shigeo, S., Tada, T., et al. (2022). Atezolizumab plus bevacizumab versus lenvatinib or sorafenib in non-viral unresectable hepatocellular carcinoma: An international propensity score matching analysis. *ESMO Open* 7 (6), 100591. PubMed PMID: 36208496. doi:10.1016/j.esmoop.2022.100591

Rizzo, A., Ricci, A. D., Gadaleta-Caldarola, G., and Brandi, G. (2021). First-line immune checkpoint inhibitor-based combinations in unresectable hepatocellular carcinoma: Current management and future challenges. *Expert Rev. gastroenterology hepatology* 15 (11), 1245–1251. PubMed PMID: 34431725. doi:10.1080/17474124.2021.1973431

Su, T. H., Wu, C. H., Liu, T. H., Ho, C. M., and Liu, C. J. (2023). Clinical practice guidelines and real-life practice in hepatocellular carcinoma: A taiwan perspective. *Clin. Mol. hepatology* 29 (2), 230–241. PubMed PMID: 36710607; PubMed Central PMCID: PMCPMC10121301. doi:10.3350/cmh.2022.0421

Wei, C. Y., Zhu, M. X., Zhang, P. F., Huang, X. Y., Wan, J. K., Yao, X. Z., et al. (2022). PKCα/ZFP64/CSF1 axis resets the tumor microenvironment and fuels anti-PD1 resistance in hepatocellular carcinoma. *J. hepatology* 77 (1), 163–176. PubMed PMID: 35219791. doi:10.1016/j.jhep.2022.02.019

Xie, D., Shi, J., Zhou, J., Fan, J., and Gao, Q. (2023). Clinical practice guidelines and real-life practice in hepatocellular carcinoma: A Chinese perspective. *Clin. Mol. hepatology* 29 (2), 206–216. PubMed PMID: 36545708; PubMed Central PMCID: PMCPMC10121293. doi:10.3350/cmh.2022.0402

Yang, C., Zhang, H., Zhang, L., Zhu, A. X., Bernards, R., Qin, W., et al. (2023). Evolving therapeutic landscape of advanced hepatocellular carcinoma. *Nat. Rev. Gastroenterology hepatology* 20 (4), 203–222. PubMed PMID: 36369487. doi:10.1038/s41575-022-00704-9

Zhang, X., Wang, J., Shi, J., Jia, X., Dang, S., and Wang, W. (2021). Cost-effectiveness of atezolizumab plus bevacizumab vs sorafenib for patients with unresectable or metastatic hepatocellular carcinoma. *JAMA Netw. Open* 4 (4), e214846. PubMed PMID: 33825837; PubMed Central PMCID: PMCPMC8027915. doi:10.1001/jamanetworkopen.2021.4846

Zhao, L., Chang, N., Shi, L., Li, F., Meng, F., Xie, X., et al. (2022). Lenvatinib plus sintilimab versus lenvatinib monotherapy as first-line treatment for advanced HBV-related hepatocellular carcinoma: A retrospective, real-world study. *Heliyon* 8 (6), e09538. PubMed PMID: 35706954; PubMed Central PMCID: PMCPMC9189019. doi:10.1016/j.heliyon.2022.e09538

Zhou, J., Sun, H., Wang, Z., Cong, W., Wang, J., Zeng, M., et al. (2020). Guidelines for the diagnosis and treatment of hepatocellular carcinoma (2019 edition). *Liver cancer* 9 (6), 682–720. PubMed PMID: 33442540; PubMed Central PMCID: PMCPMC7768108. doi:10.1159/000509424

frontiers | Frontiers in Pharmacology

TYPE Case Report
PUBLISHED 30 August 2023
DOI 10.3389/fphar.2023.1203379



OPEN ACCESS

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RECEIVED 01 May 2023
ACCEPTED 21 August 2023
PUBLISHED 30 August 2023

CITATION
Park C-R, Bae H-R, Lee G-Y, Son C-G, Cho J-H, Cho C-K and Lee N-H (2023), Feasibility of combination of Gun-Chil-Jung and cytokine-induced killer cells-based immunotherapy for terminal hepatocellular carcinoma patient: a case report.
*Front. Pharmacol.* 14:1203379.
doi: 10.3389/fphar.2023.1203379



# Feasibility of combination of Gun-Chil-Jung and cytokine-induced killer cells-based immunotherapy for terminal hepatocellular carcinoma patient: a case report

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**Introduction:** Terminal-stage hepatocellular carcinoma (HCC) is inoperable and currently has no form of adjuvant therapy. This study examined the anticancer herbal extract Gun-Chil-Jung (GCJ) combined with cytokine-induced killer (CIK)-cell-based immunotherapy as a palliative therapy for terminal HCC. We report the case of an HCC patient with extended overall survival and improved symptoms and tumor marker levels following combination therapy with GCJ and CIK cell-based immunotherapy.

**Baseline Characteristics:** From March to July 2020, a 57-year-old man who had been diagnosed with HCC underwent combination treatment with GCJ and CIK cell-based immunotherapy. By August 2021, he was prescribed GCJ. After treatment, the patient's condition was evaluated with respect to overall survival, tumor markers, symptoms, abdominal computed tomography findings, chest x-ray results, and Eastern Cooperative Oncology Group (ECOG) grade.

**Results:** The patient's overall survival, tumor marker levels, ECOG grade, and symptoms, including ascites, lower limb edema, jaundice, pleural effusion, and fatigue, were largely alleviated.

**Conclusion:** We expect that this combination therapy may be an option for palliative therapy of terminal HCC.

KEYWORDS

**case report, cytokine-induced killer cell-based immunotherapy, Gun-Chil-Jung, hepatocellular carcinoma, oncology**

## 1 Introduction

The most common type of primary liver cancer is hepatocellular carcinoma (HCC), a life-threatening disease with a poor prognosis that most often occurs in patients with chronic liver disease (Craig et al., 2020). Despite the increasing 5-year survival rate in Korea and the recently declining incidence rate of HCC, the majority of patients with

advanced-stage HCC suffers from widespread tumor distribution, including extrahepatic spread and vascular invasion, as well as decompensation of liver function (Yu, 2016). Terminal-stage HCC is usually grade 3–4 according to the Eastern Cooperative Oncology Group (ECOG) classification and class C according to the Child-Pugh score; in such cases, only symptomatic treatment is typically recommended (Barone et al., 2013).

An herbal medicine named *Gun-Chil-Jung* (GCJ) is an allergen that removes *Rhus verniciflua* Stokes (RVS) extract. Some studies about the effect of RVS extract on HCC patients have been reported previously [4.5]. There is a case report of a patient with HCC who had no feasible standard management. The progression-free survival (PFS) was over 16 months and 114 months in two advanced HCC patients, respectively, with decreased alpha-fetoprotein (AFP) levels in both after RVS treatment (Chae et al., 2018). A study of the antitumor effects of RVS antitumor effects in tumorigenic hepatocytes of mice showed that it can inhibit tumor cell growth and induce apoptosis (Son et al., 2005).

Cytokine-induced killer (CIK) cell-based immunotherapeutic agent (Immuncell-LC®; GC Cell Corp, Seoul, Korea) is an autologous immunotherapy with efficacy that has been reported in several studies (Lee et al., 2015). According to several randomized controlled trials of HCC patients receiving curative treatment, adjuvant CIK cell-based immunotherapy can reduce the recurrence rate of HCC, prevent its metastasis, and prolong overall patient survival, with very few side effects (Takayama et al., 2000; Weng et al., 2008; Hui et al., 2009; Lee et al., 2015).

Therefore, in this study, we examined the combined effect of GCJ and CIK cell-based immunotherapy for palliative care in patients with terminal HCC. In the case presented below, the patient's symptoms, tumor marker levels, and ECOG grade improved in 17 months of GCJ prescription combined with CIK-cell-based immunotherapy and, as a result, his overall survival was prolonged.

This study followed the Case Report Guidelines with the patient's informed consent and was approved by the Institutional Review Board of Daejeon University Korean medical hospital (DJUMC-2020-BM-14) (Gagnier et al., 2013).

# 2 Case presentation

## 2.1 Baseline characteristics of the patient

A 57-year-old man with ascites, lower-limb edema, jaundice, pleural effusion, and fatigue was diagnosed with terminal HCC. The disease was diagnosed as Barcelona Clinic Liver Cancer (BCLC) stage D in the BCLC Staging System and presented with poor liver function with a Child-Pugh score of C at Chungnam National University Hospital on March 8. In the absence of curative or adjuvant therapy, the patient visited the Cheonan Korean Medicine Hospital of Daejeon University for a second opinion. He had a history of skin graft surgery in Kangdong Sacred Heart Hospital in 2000, when he got burns in both legs. Furthermore, he was diagnosed with chronic hepatitis C in the same hospital and the same year. He was a non-alcoholic drinker, a non-smoker, and had no hepatitis B. According to him, he never had treatment for hepatitis C. In the computed tomography (CT) scan he brought, taken on March 8, 2020 we found that he has HCC with liver cirrhosis. Based on this history, we assumed that hepatitis C likely resulted in liver cirrhosis and HCC because he did not get proper treatment for hepatitis C. At Chungnam National University Hospital, he was notified that his HCC is cureless. Accordingly, before he visited our hospital, there were no past interventions for his HCC.

From March 20, he was administered GCJ (Supplementary Data S1) twice a day and three cycles of CIK cell-based immunotherapy (April 9 and 27, and July 10). The CIK agent was prepared at a Good Manufacturing Practice facility (GC Cell Corp, Korea) (Supplementary Data S2). Until August 2021, he had steadily received GCJ treatment twice a day. The effects of treatment were assessed based on overall survival (OS), tumor marker level, chest x-ray, abdominal CT images, and ECOG grade.

## 2.2 Overall survival and change of tumor size

As the patient expired on 15 November 2021, the overall survival was 20.3 months. The exact cause of death is unconfirmed. The survival period based on the last follow-up date (8 September 2021) was 18.3 months. In a follow-up abdominal CT conducted on December 22, there was no clear tumor progression compared with the image from July 23 and the longest diameter of the tumor decreased from 2.28 to 1.60 cm (Figure 1). Thus, the duration of response was 152 days.

## 2.3 Change in the tumor markers and liver function

The AFP level of the patient on April 27 was 2,058 ng/mL but decreased to 157 ng/mL on July 22. His serum levels of gamma-glutamyltransferase, alkaline phosphatase, and total bilirubin decreased during treatment while his alanine aminotransferase and aspartate aminotransferase levels remained unchanged. During combined GCJ prescription and CIK cell-based immunotherapy treatment, the liver function showed an improved tendency. Meanwhile, roughly a year after the last cycle of CIK cell-based immunotherapy, the overall liver function test levels increased again (Figure 2).

## 2.4 Ascites and pleural effusion

Because of ascites and pleural effusion, prior to combination therapy, the patient frequently underwent paracentesis, despite taking a regularly prescribed diuretic. After combination therapy, the ascites accumulated more slowly, resulting in a decrease in the patient's abdominal circumference from 96 to 87 cm. Abdominal CT conducted on July 23 indicated ascites shrinkage compared to the image obtained on March 8 (Figure 3). Even after the patient stopped taking diuretics from July 27, follow-up chest x-rays conducted on October 23 and December 10 showed a reduction in pleural effusion compared with the image from July 10

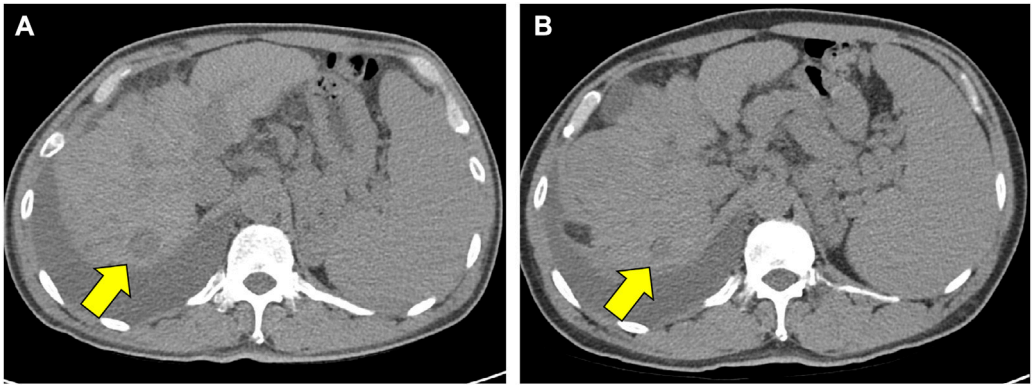


FIGURE 1
Decrease of tumor size in the CT scan images. **(A)** Is an abdomen CT image which were scanned on July 23 **(B)** is a follow-up abdomen CT image which were scanned on December 22. Yellow arrows indicate a decrease in the longest diameter of tumor mass from 2.28 cm in **(A)**, to 1.60 cm in **(B)**. Abbreviations: CT, computed tomography.

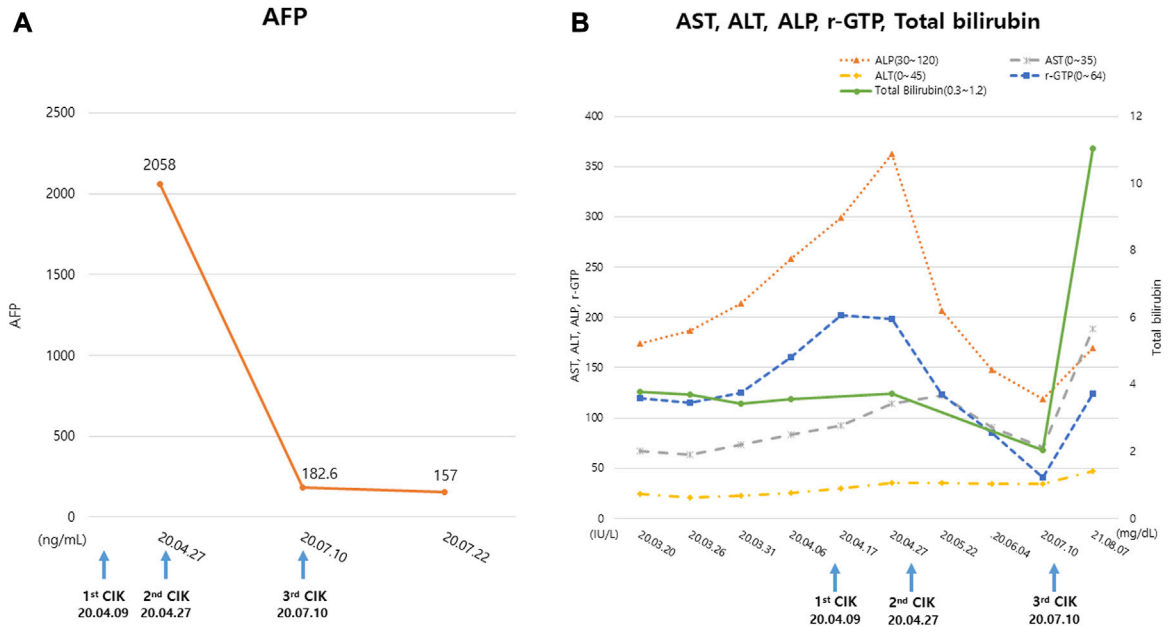


FIGURE 2
Change in the tumor markers and liver function test. **(A)** The tumor marker, AFP level examined during the treatment period **(B)** The serum ALT, AST, ALP, r-GTP, Total bilirubin levels examined during the treatment period. The blue arrows indicate the date of CIK cell-based immunotherapy administration. 'CIK' means CIK cell-based immunotherapy. Abbreviations: AFP, alpha-fetoprotein; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CIK, Cytokine-induced killer; r-GTP, gamma glutamyl transpeptidase.

(Figure 4). The patient was still alive and relatively healthy without needing a paracentesis or diuretics, until August 2021, approximately 3 months before he expired.

## 2.5 Physical performance

Based on these changes, the patient's ECOG grade decreased from 3 to 2. He was able to engage in social activities and both his physical strength and appetite improved.

## 2.6 Adverse event and safety

During the treatment process, no serious adverse events occurred. Chemotherapy or small-molecule inhibitors usually cause adverse events such as fatigue, diarrhea, and reduced quality of life (Kamal et al., 2022). Compared with standard treatment options for advanced HCC, GCJ combined with CIK cell-based immunotherapy has safety benefits.

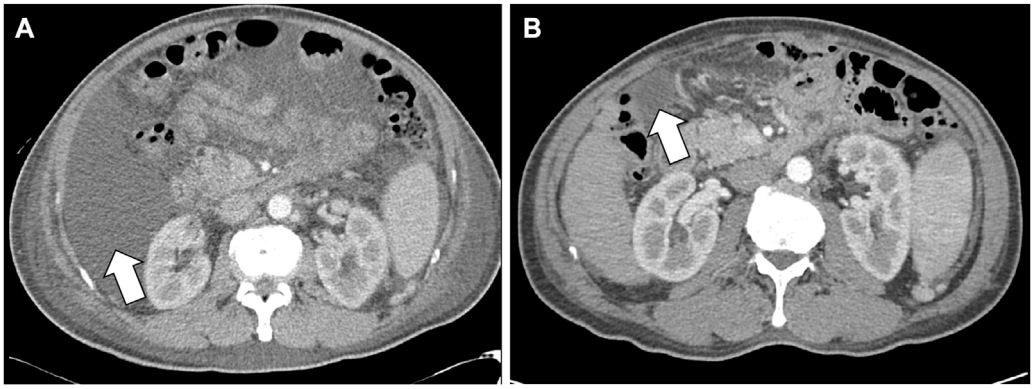


FIGURE 3
Decrease of ascites in the CT scan images. **(A)** Is an abdomen CT image which were scanned on March 8 **(B)** is a follow-up abdomen CT image which were scanned on July 23. White arrows indicate a shrinkage of ascites in **(B)**, compared to **(A)**. Abbreviations: CT, computed tomography.

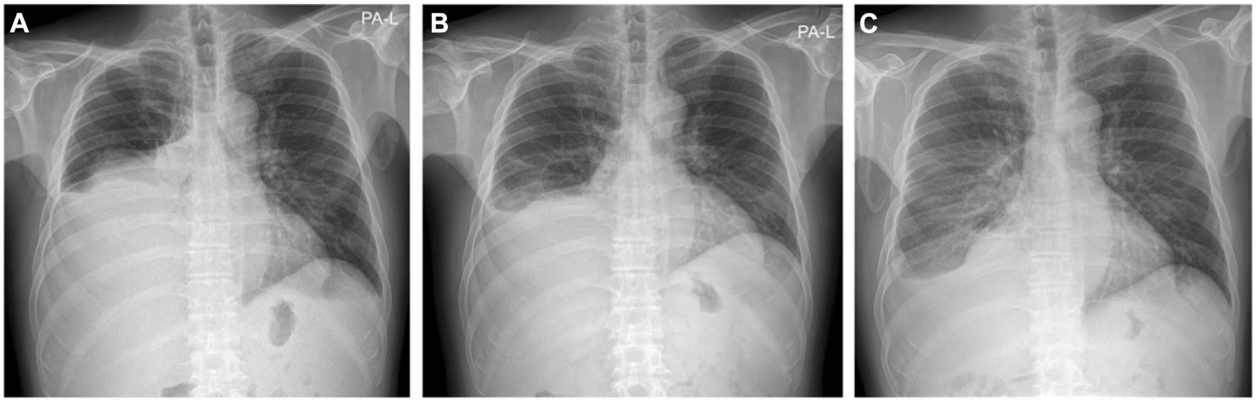


FIGURE 4
Chest X-ray images. **(A)** Chest X-ray on July 10 **(B)** Chest X-ray on October 23 **(C)** Chest X-ray on December 10. Even after he stopped taking diuretic from July 27, the chest X-ray findings above show decrease of pleural effusion.

## 3 Discussion

Despite the increase in first-line treatment options including Atezolizumab–Bevacizumab, Sorafenib, and Lenvatinib, terminal HCC treatment cannot cure HCC. It aims to control the cancer, relieve its symptoms, and give patients a good quality of life. In a meta-analysis, the survival of patients with terminal HCC was estimated at a 1-year survival rate of 11% (95% CI, 4.7–22; range, 0%–57%) (Cabibbo et al., 2010). Terminal HCC should thus be managed with palliative support, including nutritional supplements, pain control, and psychological assistance (Kumar and Panda, 2014).

While immunotherapies have made great strides in the fight against HCC, single immunotherapy has shown that a high percentage of patients still fail to respond and that tumors have the potential to become resistant to terminal HCC. Therefore, this study focused on the potential of combining GCJ with CIK-cell-based immunotherapy to improve the response rates and long-term outcomes of patients.

Our patient had terminal HCC, for which he received palliative care with GCJ and CIK cell-based immunotherapy. The efficacy of these two therapies for HCC has been reported in previous studies (Takayama et al., 2000; Wang et al., 2002; Son et al., 2005; Weng et al., 2008; Hui et al., 2009; Kim et al., 2010; Lee et al., 2015; Chae et al., 2018). RVS extract is an anticancer substance that promotes cancer cell apoptosis, suppresses cancer cell growth, and inhibits angiogenesis (Choi et al., 2012). Some studies have demonstrated its effect on various kinds of cancers. For example, in a clinical study with 40 non-small cell lung cancer (NSCLC) patients, oral administration of RVS extract prolonged OS and PFS rate (Cheon et al., 2011). In a case study about a gastric cancer patient, the tumor shrank after 5 months of treatment with orally administered RVS extract (Lee et al., 2010). Moreover, an *in vitro* study using biliary tract cancer cells shows that RVS extract downregulates the proliferation and upregulates the apoptosis of cancer cells (Joung and Kim, 2015). Another *in vitro* study with breast cancer cells demonstrated that RVS treatment induces cancer apoptosis through the Adenosine monophosphate (AMP)-activated

protein kinase signaling pathway (Lee et al., 2014). The main compounds of GCJ, an herbal extract of RVS, are fisetin, fustin, and sulfuretin, all of which have apoptotic actions in diverse types of cancer (Moon et al., 2015; Jun et al., 2020). Fisetin is an apoptotic component for prostate, pancreatic, and colon cancer cells (Khan et al., 2008; Suh et al., 2008; Murtaza et al., 2009). Sulfuretin also induces apoptosis in leukemia cells through the Fas-mediated caspase-8–dependent pathway, which activates apoptotic factors (Lee et al., 2012). GCJ has traditionally been used to relieve blood stasis and promote detoxification (Yoo and Roh, 1977). The benefits of RVS extract in a patient with post-liver transplantation recurrent HCC and lung metastasis have been described in a case study (Kim et al., 2010). The positive effects include prolonged survival and the shrinkage of the metastatic region of the lung (Kim et al., 2010).

CIK cell-based immunotherapy consists of a mixture of T lymphocytes comprising $CD3^+/CD56^+$ cells, $CD3^+/CD56^-$ cytotoxic T cells, and $CD3^-/CD56^+$ natural killer cells. The mixture was prepared from the patient's peripheral blood, and mononuclear cells inside the blood were cultured *ex vivo* through co-stimulation with the anti-CD3 antibody and interleukin-2 (Lee et al., 2015). The antitumor cytotoxic activity and tumor growth inhibition of CIK cells in HCC have been examined both *in vitro* and *in vivo* (Wang et al., 2002). CIK cells are estimated to be involved in eliminating HCC cells, likely through interactions with leukocyte function-associated antigen-1, which is related to cytolysis in HCC target cells (Wang et al., 2002). Moreover, significantly improved OS and recurrence- or progression-free survival were shown in numerous trials including advanced HCC (Zhang and Schmidt-Wolf, 2020; Han et al., 2022).

The combined use of GCJ and CIK cell-based immunotherapy may improve immune function in tumors. The mild adverse events and multiple improvements in their anti-cancer activity make GCJ and CIK-cell-based immunotherapy a favorable therapeutic option in cancer immunotherapy. Combining herbal medicine and adoptive cell therapy decreased tumor markers, such as alpha-fetoprotein (AFP), and improved immune functions, such as those involving $CD3^+$, while increasing $CD3^+CD56^+$ and $CD3^+CD8^+$ cell ratios in the peripheral blood. This indicates that continued decreases in AFP concentrations after CIK cell therapy may be the pathway via which CIK and GCJ exert their roles in preventing short-term progression, which can be used to predict the clinical efficacy of CIK-based immunotherapy as a form of maintenance treatment for patients with terminal HCC. In this study, in approximately 17 months of GCJ treatment combined with CIK cell-based immunotherapy, the patient's outcome was favorable, as indicated by better performance status and decreased ascites, pleural effusion, and tumor marker levels. Liver function levels improved when CIK cell-based immunotherapy was combined with GCJ, although it aggravated after the last cycle of the therapy ended. This indicates that GCJ treatment may be more effective when combined with CIK cell-based immunotherapy.

As a result, the patient's overall survival extended to 20.3 months. This is an encouraging outcome compared to the median survival period of terminal HCC patients of three to four months (Cabibbo et al., 2010). This suggests that GCJ is a promising candidate for anti-cancer drugs with a gamut of therapeutic applications.

The limitations of this case are as follows: First, although this case has achieved long-term response duration, it is not universally representative. Second, the combined effect requires further clarification. Third, the patient was followed up retrospectively, and the pharmacokinetic/pharmacodynamic activity of T cells could not be accurately detected. With further research, the mechanism of combination immunotherapy should be further explored.

## 4 Conclusion

This case report demonstrates the utility of combination treatment with GCJ and CIK cell-based immunotherapy to extend OS and improve tumor marker levels, tumor-related symptoms, and ECOG grade in a patient with terminal HCC. This study reported a favorable therapeutic effect on patients; immunotherapy may be a potentially feasible systemic treatment for terminal cancers that cannot be cured or treated. Clinical trials and systematic studies with a sufficient number of patients are needed to further corroborate these results.

## Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author.

## Ethics statement

The studies involving humans were approved by the Institutional Review Board of Daejeon University Korean medical hospital (DJUMC-2020-BM-14). The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

## Author contributions

C-RP and N-HL contributed to conception and design of the study. H-RB and G-YL performed the statistical analysis. C-RP and N-HL wrote the first draft of the manuscript. H-RB, G-YL, C-GS, J-HC, and C-KC wrote sections of the manuscript. All authors contributed to the article and approved the submitted version.

## Funding

This work was supported by a grant from the National Research Foundation of Korea (NRF) (No. RS-2023-00246351).

## Acknowledgments

The authors thank GC Cell Corp for providing assistance on the research of CIK agent.

## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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## Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphar.2023.1203379/full#supplementary-material

**SUPPLEMENTARY FIGURE S1**
Fingerprinting analysis of GCJ components. **(A)** Fingerprinting analysis of standard solution: **(B)** Fingerprinting analysis of GCJ: **(C)** Contents of GCJ components. Abbreviations: GCJ, Gun-Chil-Jung.

## References

Barone, C., Koeberle, D., Metselaar, H., Parisi, G., Sansonno, D., and Spinzi, G. (2013). Multidisciplinary approach for HCC patients: hepatology for the oncologists. *Ann. Oncol.* 24 (2), ii15–ii23. doi:10.1093/annonc/mdt053

Cabibbo, G., Enea, M., Attanaio, M., Bruix, J., Craxi, A., and Camma, C. (2010). A meta-analysis of survival rates of untreated patients in randomized clinical trials of hepatocellular carcinoma. *Hepatology* 51, 1274–1283. doi:10.1002/hep.23485

Chae, J., Lee, S., and Lee, S. (2018). Potential efficacy of allergen removed Rhus verniciflua Stokes extract to maintain progression-free survival of patients with advanced hepatobiliary cancer. *Explore* 14 (4), 300–304. doi:10.1016/j.explore.2017.10.013

Cheon, S. H., Kim, K. S., Kim, S., Jung, H. S., Choi, W. C., and Eo, W. K. (2011). Efficacy and safety of Rhus verniciflua Stokes extracts in patients with previously treated advanced non-small cell lung cancer. *Forsch Komplementmed* 18 (2), 77–83. doi:10.1159/000327306

Choi, W., Jung, H., Kim, K., Lee, S., Yoon, S., Park, J., et al. (2012). Rhus verniciflua Stokes against advanced cancer: a perspective from the Korean integrative cancer center. *J. Biomed. Biotechnol.* 2012, 874276. doi:10.1155/2012/874276

Craig, A. J., von Felden, J., Garcia-Lezana, T., Sarcognato, S., and Villanueva, A. (2020). Tumour evolution in hepatocellular carcinoma. *Nat. Rev. Gastroenterol. Hepatol.* 17 (3), 139–152. doi:10.1038/s41575-019-0229-4

Gagnier, J., Kienle, K., Altman, D., Moher, D., Sox, H., Riley, D., et al. (2013). The CARE guidelines: consensus-based clinical case reporting guideline development. *Glob. Adv. Heal Med.* 2, 38–43. doi:10.7453/gahmj.2013.008

Han, M. H., Kim, J. M., Cheong, J. H., Ryu, J. I., Won, Y. D., Nam, G. H., et al. (2022). Efficacy of cytokine-induced killer cell immunotherapy for patients with pathologically pure glioblastoma. *Front. Oncol.* 12, 851628. doi:10.3389/fonc.2022.851628

Hui, D., Qiang, L., Jian, W., Ti, Z., and Da-Lu, K. (2009). A randomized, controlled trial of postoperative adjuvant cytokine-induced killer cells immunotherapy after radical resection of hepatocellular carcinoma. *Dig. Liver Dis.* 41 (1), 36–41. doi:10.1016/j.dld.2008.04.007

Joung, B. B., and Kim, Y. C. (2015). Study on anti-cancer effects of Rhus verniciflua Stokes extracted with sterile distilled water on two cholangiocarcinoma cell lines, SNU-1079 and SNU-1196. *J. Int. Korean Med.* 36 (1), 1–12.

Jun, H. J., Park, S. J., Kang, H. J., Lee, G. Y., Lee, N., et al. (2020). The survival benefit of combination therapy with mild temperature hyperthermia and an herbal prescription of gun-chil-jung in 54 cancer patients treated with chemotherapy or radiation therapy: a retrospective study. *Integr. Cancer Ther.* 19, 1534735420926583–12. doi:10.1177/1534735420926583

Kamal, M. A., Mandour, Y. M., Abd El-Aziz, M. K., Stein, U., and El Tayebi, H. M. (2022). Small molecule inhibitors for hepatocellular carcinoma: advances and challenges. *Molecules* 27 (17), 5537. doi:10.3390/molecules27175537

Khan, N., Afaq, F., Syed, D. N., and Mukhtar, H. (2008). Fisetin, a novel dietary flavonoid, causes apoptosis and cell cycle arrest in human prostate cancer LNCaP cells. *Carcinogenesis* 29, 1049–1056. doi:10.1093/carcin/bgn078

Kim, H. R., Kim, K. S., Jung, H. S., Choi, W. C., Eo, W. K., and Cheon, S. H. (2010). A case of recurred hepatocellular carcinoma refractory to doxorubicin after liver transplantation showing response to herbal medicine product, Rhus verniciflua Stokes extract. *Integr. Cancer Ther.* 9 (1), 100–104. doi:10.1177/1534735409359772

Kumar, M., and Panda, D. (2014). Role of supportive care for terminal stage hepatocellular carcinoma. *J. Clin. Exp. Hepatology* 4 (3), S130–S139. doi:10.1016/j.jceh.2014.03.049

Lee, J. H., Lee, J. H., Lim, Y. S., Yeon, J. E., Song, T. J., Yu, S. J., et al. (2015). Adjuvant immunotherapy with autologous cytokine-induced killer cells for hepatocellular carcinoma. *Gastroenterology* 148 (7), 1383–1391. doi:10.1053/j.gastro.2015.02.055

Lee, J. O., Moon, J. W., Lee, S. K., Kim, S. M., Kim, N., Ko, S. G., et al. (2014). Rhus verniciflua extract modulates survival of MCF-7 breast cancer cells through the modulation of AMPK-pathway. *Biol. Pharm. Bull.* 37 (5), 794–801. doi:10.1248/bpb.b13-00893

Lee, K. W., Chung, K. S., Seo, J. H., Yim, S. V., Park, H. J., Choi, J. H., et al. (2012). Sulfuretin from heartwood of *Rhus verniciflua* triggers apoptosis through activation of Fas, Caspase-8, and the mitochondrial death pathway in HL-60 human leukemia cells. *J. Cell Biochem.* 113, 2835–2844. doi:10.1002/jcb.24158

Lee, S. H., Choi, W. C., Kim, K. S., Park, J. W., Lee, S. H., and Yoon, S. W. (2010). Shrinkage of gastric cancer in an elderly patient who received Rhus verniciflua Stokes extract. *J. Altern. Complement. Med.* 16 (4), 497–500. doi:10.1089/acm.2008.0237

Moon, J. E., Kim, D. M., and Kim, J. Y. (2015). Anti-inflammatory effect of Rhus verniciflua Stokes extract in the murine macrophage cell line, Raw264.7. *J. Korean Soc. Appl. Biol. Chem.* 58 (4), 481–486. doi:10.1007/s13765-015-0065-3

Murtaza, I., Adhami, V. M., Hafeez, B. B., Saleem, M., and Mukhtar, H. (2009). Fisetin, a natural flavonoid, targets chemoresistant human pancreatic cancer AsPC-1 cells through DR3-mediated inhibition of NF-kappaB. *Int. J. Cancer* 125, 2465–2473. doi:10.1002/ijc.24628

Son, Y. O., Lee, K. Y., Lee, J. C., Jang, H. S., Kim, J. G., Jeon, Y. M., et al. (2005). Selective antiproliferative and apoptotic effects of flavonoids purified from Rhus verniciflua Stokes on normal versus transformed hepatic cell lines. *Toxicol. Lett.* 155 (1), 115–125. doi:10.1016/j.toxlet.2004.09.003

Suh, Y., Afaq, F., Johnson, J. J., and Mukhtar, H. (2008). A plant flavonoid fisetin induces apoptosis in colon cancer cells by inhibition of COX2 and Wnt/EGFR/NF-kappaB-signaling pathways. *Carcinogenesis* 30, 300–307. doi:10.1093/carcin/bgn269

Takayama, T., Sekine, T., Makuuchi, M., Yamasaki, S., Kosuge, T., Yamamoto, J., et al. (2000). Adoptive immunotherapy to lower postsurgical recurrence rates of hepatocellular carcinoma: a randomised trial. *Lancet* 356 (9232), 802–807. doi:10.1016/S0140-6736(00)02654-4

Wang, F. S., Liu, M. X., Zhang, B., Shi, M., Lei, Z. Y., Sun, W. B., et al. (2002). Antitumor activities of human autologous cytokine-induced killer (CIK) cells against hepatocellular carcinoma cells *in vitro* and *in vivo*. *World J. Gastroenterol.* 8 (3), 464–468. doi:10.3748/wjg.v8.i3.464

Weng, D. S., Zhou, J., Zhou, Q. M., Zhao, M., Wang, Q. J., Huang, L. X., et al. (2008). Minimally invasive treatment combined with cytokine-induced killer cells therapy lower the short-term recurrence rates of hepatocellular carcinomas. *J. Immunother.* 31 (1), 63–71. doi:10.1097/CJI.0b013e31815a121b

Yoo, H., and Roh, J. (1977). *Compendium of prescriptions from the countryside (hyangyakjipseongbang;* 鄕藥集成方*)*. Seoul, Republic of Korea: Hangrimchulpan.

Yu, S. J. (2016). A concise review of updated guidelines regarding the management of hepatocellular carcinoma around the world: 2010-2016. *Clin. Mol. Hepatol.* 22 (1), 7–17. doi:10.3350/cmh.2016.22.1.7

Zhang, Y., and Schmidt-Wolf, I. G. H. (2020). Ten-year update of the international registry on cytokine-induced killer cells in cancer immunotherapy. *J. Cell Physiol.* 235 (12), 9291–9303. doi:10.1002/jcp.29827

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