THE LANDSCAPE OF COMPREHENSIVE THERAPY BASED ON SYSTEMIC THERAPY FOR UNRESECTABLE HEPATOCELLULAR CARCINOMA (uHCC) IN CHINA

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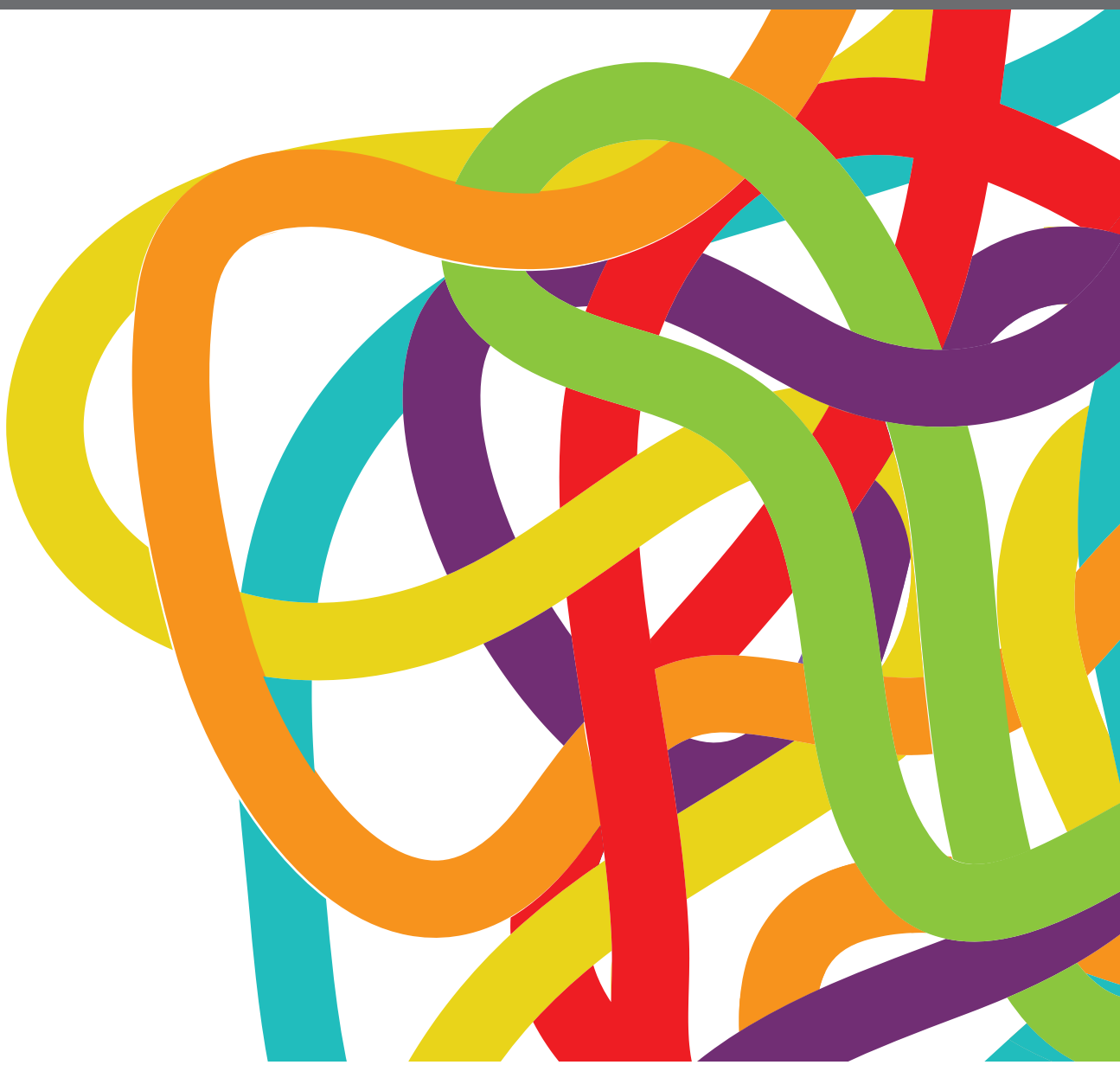

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THE LANDSCAPE OF COMPREHENSIVE THERAPY BASED ON SYSTEMIC THERAPY FOR UNRESECTABLE HEPATOCELLULAR CARCINOMA (uHCC) IN CHINA

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Elevated DNA Polymerase Delta 1 Expression Correlates With Tumor Progression and Immunosuppressive Tumor Microenvironment in Hepatocellular Carcinoma

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Background and Objective: Hepatocellular carcinoma (HCC) is one of the most common cancers worldwide, and the DNA polymerase delta (POLD) family is significantly related to cancer prognosis. This study aimed to explore the significance of the POLD family in HCC *via* the DNA damage repair (DDR) pathway.

Methods: Data mining was conducted using bioinformatics methods. RNA sequencing and clinicopathological data were collected from The Cancer Genome Atlas, GTEx database and the Gumz Renal cohort. Statistical analyses were also performed in cancer samples (n>12,000) and the Affiliated Hospital of Youjiang Medical University for Nationalities (AHYMUN, n=107) cohort.

Results: The POLD family (POLD1–4) was identified as the most important functional component of the DDR pathway. Based on the analysis of independent cohorts, we found significantly elevated POLD expression in HCC compared with normal tissues. Second, we investigated the prognostic implication of elevated POLD1 expression in HCC and pan-cancers, revealing that increased POLD1 levels were correlated to worse prognoses for HCC patients. Additionally, we identified 11 hub proteins interacting closely with POLD proteins in base excision repair, protein-DNA complex and mismatch repair signaling pathways. Moreover, POLD1 mutation functioned as an independent biomarker to predict the benefit of targeted treatment. Importantly, POLD1 expression was associated with immune checkpoint molecules, including CD274, CD80, CD86, CTLA4, PDCD1 and

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TCGIT, and facilitated an immune-excluded tumor microenvironment. Additionally, we confirmed that elevated POLD1 expression was closely correlated with the aggressive progression and poor prognosis of HCC in the real-world AHYMUN cohort.

Conclusion: We identified a significant association between elevated POLD1 expression and poor patient survival and immune-excluded tumor microenvironment of HCC. Together, these findings indicate that POLD1 provides a valuable biomarker to guide the molecular diagnosis and development of novel targeted therapeutic strategies for HCC patients.

Keywords: POLD1, hepatocellular carcinoma, prognosis, immune microenvironment, biomarker

INTRODUCTION

Hepatocellular carcinoma (HCC) is the most common type of primary liver cancer, with over half a million new cases diagnosed worldwide each year. HCC is the most malignant form of liver cancer, which derives from the aggressive proliferation of liver epithelial cells (1). As the sixth leading cause of cancer-related mortality globally, HCC accounts for 4.7% of all cancer deaths. In 2020, over 910,000 people were diagnosed with HCC, and its incidence is continuously rising (2). In China, the incidence of HCC is particularly high, accounting for 55% of the total number of HCC patients worldwide.

Several molecular pathways are implicated in HCC carcinogenesis, including vascular endothelial growth factor receptor (VEGFR), TP53 and Akt/mTOR pathways (3). Among several pathogenic factors, cirrhosis underlies most cases of HCC, and hepatitis C virus (HCV) infection also causes cirrhosis and HCC. However, the eradication of HCV-related cirrhosis does not prevent the development of HCC (4, 5). Thus, more effective HCC treatments are needed.

Historically, serum alpha-fetoprotein (AFP) and diagnostic imaging were the primary diagnostic methods for HCC. However, the poor prognosis of patients owing to the late diagnosis of HCC is unacceptable, and AFP levels are not significantly elevated in most small and early-stage HCCs (6). Recently, several studies have focused on identifying promising biomarkers for early detection (7, 8). Currently, targeted drugs, such as sorafenib, are used as a first-line treatment strategy for the personalized treatment of advanced HCC (9). However, exploring unknown targets and pathways is important to achieve more significant survival benefits for advanced HCC patients who cannot be cured through surgery.

DNA damage repair (DDR) pathways consist of multiple interconnected cellular signaling networks that are activated in response to DNA damage. DDR is correlated with genomic instability, tumor mutational burden in HCC and immune cell function (10). Several studies have shown that the development of cancer is mainly driven by defects in DDR. Therefore, inhibitors targeting kinases involved in DDR, such as AZ20 and M3814, have been investigated for the treatment of cancers in clinical trials (11).

The DNA polymerase delta (POLD) family consisting of POLD1, POLD2, POLD3 and POLD4 is an important mediator of DNA repair during chromosome replication, which is of great significance to the maintenance of DNA structure stability (12). *POLD1* encodes the catalytic subunit of DNA polymerase delta, which is involved in both polymerase and 3' to 5' exonuclease activities. POLD1 plays a crucial role in DNA replication and DNA double-strand break repair, together with the accessory proteins POLD2, POLD3 and POLD4 for full activity (13, 14). POLD2 encodes the 50-kDa catalytic subunit of DNA polymerase delta, assisting POLD1 in the process of DDR.

In addition, POLD3 and POLD4 may catalyze the repair of damaged replication forks *via* break-induced replication (15). Recent studies have shown that the loss or reduced expression of POLD3 predicts the occurrence and progression of colorectal cancer and other malignant tumors (16, 17). The decreased expression of POLD3 is associated with the pathogenesis of HCC by enhancing the proliferation and invasion abilities of tumor cells (18).

However, the relationship between the expression of POLD family members and the prognosis of HCC remains poorly understood. Based on the role of the POLD family in the maintenance and repair of DNA, we hypothesized that POLD expression significantly affects the prognosis of HCC *via* the DDR pathway. Our study aims to identify a biomarker with significant potential to improve the diagnosis and prognosis of HCC.

METHODS

Patients and Tissue Samples From Online Databases and Real-World Cohorts

The large-scale cohorts in this study included RNA sequencing data and accompanying clinicopathological data for 423 HCC patients collected from The Cancer Genome Atlas (TCGA, http://www.cancer.gov) and 10 patients with HCC from the Gumz Renal cohort in the Oncomine (http://www.oncomine.com) database. The threshold was set as follows: *P*-value<0.05, gene rank>10% and data type: mRNA, Cancer *vs*. Normal.

The Affiliated Hospital of Youjiang Medical University for Nationalities (AHYMUN, Guangxi, China) cohort consisted of 107 patients diagnosed with HCC in the Department of Hepatology, Affiliated Hospital of Youjiang Medical University for Nationalities, from June 2009 to August 2018. Clinicopathological data were collected from pathology reports

or electronic medical records. Samples of HCC and normal liver tissues were collected during surgery and then processed and stored at the AHYMUN tissue bank before experiments.

Immunohistochemistry (IHC) Staining Analysis

IHC was performed with an anti-POLD1 antibody-N-terminal (ab226848, Abcam) at a 1:500 dilution. IHC staining was conducted in accordance with the manufacturer's instructions as previously described (19). Based on the IHC staining intensity and density, two experienced and independent pathologists/clinicians evaluated the overall IHC score (from 0 to 12), with a score of 0 to 3 indicating negative staining and a score of 4 to 12 indicating positive staining for each tissue.

Establishment of a Protein-Protein Interaction (PPI) Network and Functional Annotations of POLD-Related PPI Networks

In this study, an online web tool for the retrieval of interacting genes (STRING, http://string-db.org; version 10.0) was used to establish a PPI network of differentially expressed genes (DEGs) related to POLD. Then, Spearman's correlation analysis was performed to describe the correlation between quantitative variables without a normal distribution. The gene ontology (GO) database was used for functional enrichment analyses of biological processes (BP), molecular functions (MF) and cellular components (CC). Kyoto Encyclopedia of Genes and Genomes (KEGG) and Reactome pathway enrichment databases were used to assess large-scale biologic molecular datasets. The publicly available WEB-based GEne SeT AnaLysis Toolkit (WebGestalt; http://www.webgestalt.org/; Version 6.8) was used to explore the potential function of the POLD-related gene panel (20).

Abundance and Frequency of POLD Mutations in HCC

To further investigate the role of POLD in HCC and pan-cancers, we analyzed the abundance and frequency of POLD mutations in HCC using cBioportal for cancer genomics (http://www.cbioportal.org/). The frequency of typical gene mutations in HCC according to differential POLD1 expression was also analyzed. Significantly elevated genes between POLD1 altered and unaltered groups were screened and identified using the Limma R package.

Analysis of Immune Infiltration in the Tumor Microenvironment

We grouped the HCC patients (n=107, AHYMUN cohort) into $POLD1^{high}$ and $POLD1^{low}$ groups using the median POLD expression. Tumor Immune Estimation Resource 2.0 (TIMER 2.0, http://timer.cistrome.org/) was used to analyze the correlation between the abundance of tumor-infiltrating immune cells and POLD expression using Spearman's test. Then, R software was used to evaluate the interactions between immune checkpoint molecules and tumor-infiltrating lymphocytes (TILs) in human cancer samples from TCGA datasets. Spearman's test was also used to determine the relationship between POLD1 expression and tumor purity using the ESTIMATE algorithm (21).

Statistics Analysis

To determine the statistical significance of differential POLD expression between tumor and normal tissues, a Student's t-test was performed. Kaplan–Meier curves with their 95% confidence intervals (95%CIs) and log-rank tests were applied to evaluate the significance of disease-specific survival (DSS) and overall survival (OS) benefits in separate POLD expression groups and all subgroups classified by tumor microenvironment infiltration characteristics using the Kaplan–Meier Plotter (http://kmplot.com/analysis/index). Univariate and multivariate Cox regression analyses were performed to identify the proper terms to build the nomogram. A forest plot was generated with the "forestplot" R package and used to show the *P*-value, hazard ratio (HR) and 95% CI of each variable. A nomogram was developed based on the results of multivariate Cox proportional hazard analysis to predict the X-year overall recurrence and calculate the risk of recurrence for individual patients. Moreover, a Sankey diagram was used to explore the relationship between pathological factors and patient survival.

All statistical analyses were performed using SPSS software (version 23.0, Inc, Chicago, IL), GraphPad Prism 8.0, R software (version 3.4.3), or online web tools. All hypothetical analyses were two-sided, and $P<0.05$ was considered statistically significant.

RESULTS

Identification of Critical DDR Pathway Genes and Their Prognostic Implications in HCC

This study was conducted in three stages (**Figure S1**). To determine the most significant genes in the DDR pathway, we first screened genes involved in base excision repair, nucleotide excision repair, mismatch repair, homologous recombination, non-homologous end-joining and Fanconi anemia pathways. We arranged these genes according to the number of pathways they are involved in and found that the POLD family participated in four pathways, indicating the important value of POLDs in DDR (**Figure 1A**). Next, the differential expression in POLD family members between >12,000 tumor and normal tissues was assessed. The expression level of POLDs was significantly higher in HCC tumor tissues compared with normal tissues (**Figure 1B**). Furthermore, POLD1 had the highest Z-score in the heatmap compared with POLD2, POLD3 and POLD4, indicating its greater significance for HCC prognosis (**Figure 1C**). Based on these results, we mainly explored the important role of POLDs in DDR and the effect of POLD expression on the development and prognosis of HCC.

Prognostic Role of POLDs in HCC

To further confirm the research direction, we performed Kaplan–Meier analysis of 364 patients from the TCGA cohort.

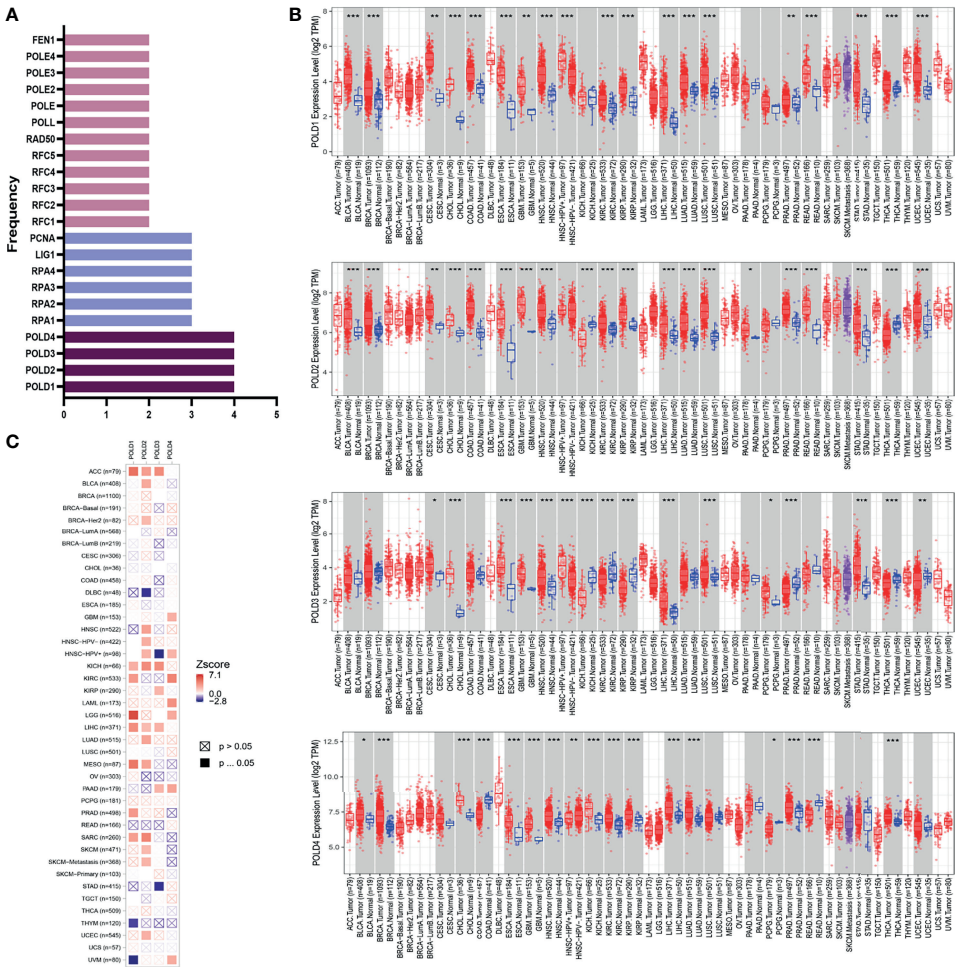

FIGURE 1 | Identification of POLD family members as significant factors in DDR pathways and their prognostic implication in cancer. **(A)** Genes involved in base excision repair, nucleotide excision repair, mismatch repair, homologous recombination, non-homologous end-joining and Fanconi anemia pathways were evaluated, and POLD family members were identified in all four pathways. **(B)** The expression levels of POLDs were significantly higher in HCC tumor tissues compared with normal tissues in more than 12,000 samples. *p < 0.05, **p < 0.01, ***p < 0.001. **(C)** Heatmap analysis indicated that POLD1 has the most significant influence on HCC prognosis compared with POLD2, POLD3 and POLD4.

High POLD1 expression remarkably predicted a worse OS of HCC patients, whereas patients with higher expression of POLD2, POLD3 and POLD4 did not exhibit significantly shorter OS compared with lower expression patients (**Figures 2A–D**). Then, we conducted subgroup survival analyses of POLD1 expression based on hepatitis virus infection, clinicopathological staging and sorafenib use in 364 patients with HCC (**Figures 2E–H**). The results suggested that elevated POLD1 expression significantly predicted poor OS in the hepatitis virus infected and uninfected subgroup. However, for HCC patients with an advanced stage, POLD1 expression was not significantly associated with prognosis (HR=1.71, *P*=0.13) but predicted significantly worse outcomes for those receiving sorafenib treatment (HR=4.13, *P*=0.026). Additionally, high POLD1 expression was remarkably related to poor PFS in 370 HCC patients (**Figure 2I**).

POLD1 Significantly Predicts Survival and Aggressive Clinicopathological Parameters for HCC Patients

Next, we explored the relationship between the expression of POLD1 and the survival of HCC patients. HCC patients with higher POLD1 expression experienced a significantly increased risk of death, and the z-score of POLD1 expression confirmed that elevated POLD1 levels were associated with higher mortality (**Figure 3A**). The Kaplan–Meier curve also demonstrated that high POLD1 expression led to worse OS in 371 patients, with a median survival of 2.8 years in the $POLD^{high}$ group and 6.3 years in the $POLD^{low}$ group (**Figure 3B**). The high sensitivity and specificity of the independent diagnostic and prognostic value of POLD1 expression were shown by the ROC curve (**Figure 3C**). However, the accuracy decreased over time [1-year area under the curve (AUC)=0.742, 3-year AUC=0.666 and 5-year

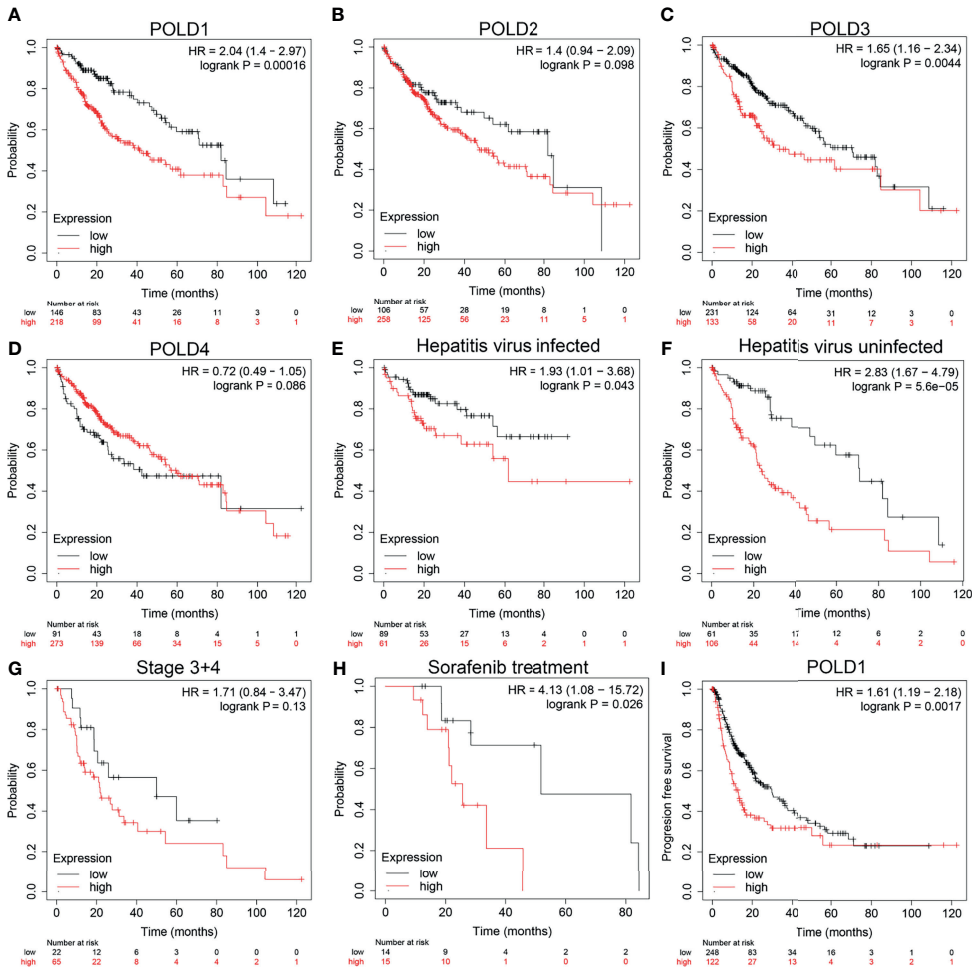

FIGURE 2 | High_ expression of POLD1 is associated with poor survival of HCC patients. **(A–D)** High expression of POLD1 predicted poor OS of HCC patients. The expressions of POLD2, POLD3 and POLD4 had no influence on OS. **(E–H)** Analysis of subgroups (hepatitis virus infection, clinicopathological staging and sorafenib use) in 364 HCC patients from a TCGA cohort indicated that high POLD1 expression was significantly_ linked with poor prognosis. **(I)** High expression of POLD1 was connected with poor PFS in HCC patients (n = 370).

AUC=0.610], suggesting that POLD1 expression more accurately predicted the prognosis of early-stage HCC patients compared with those with advanced stages.

Next, we assessed the Gumz Liver cohort consisting of differential RNA-seq data from HCC and normal samples. The results revealed a significantly elevated POLD1 expression level in HCC compared with normal samples (n=22; *P*=0.043; **Figure 4A**). The Sankey diagram shows the relationships between tumor grades, stages, POLD1 expression levels and survival. Low-tumor grade and early-stage HCC patients tended to have reduced expression of POLD1, whereas high tumor grades and advanced stages were related to the increased POLD1 expression (**Figure 4B**).

Differential Expression of POLD1 in Pan-Cancers and Its Prognostic Value

Based on HCC tissues and adjacent normal tissues from TCGA (n=423) and GTEx (n=533) datasets, we first compared the POLD1 expression level between pan-cancers and normal tissues. Significant differential expression of POLD1 was commonly observed between cancer tissues and adjacent normal tissues in pan-cancers, such as GMB and STAD, and the expression of POLD1 was significantly higher in the HCC tumor group compared with the normal group (**Figures S2A, B**). Second, to explore the prognostic implications of POLD1 expression in pan-cancers, we performed Cox regression analysis (**Figures S2C, D**). POLD1 expression was closely correlated with OS in several cancers, such as PRAD (HR=9.25; *P*=2.7e-04) and MESO (HR=2.71; *P*=1.7e-06), and DSS in KIRC (HR=1.93; *P*=7.4e-04) and ACC (HR=3.48; *P*=2.6e-06).

Construction of a Prediction Model Using Cox Regression and Nomogram Analysis

The univariate Cox regression analysis suggested that POLD1, POLD2, POLD3 and pTNM stages were closely related to the

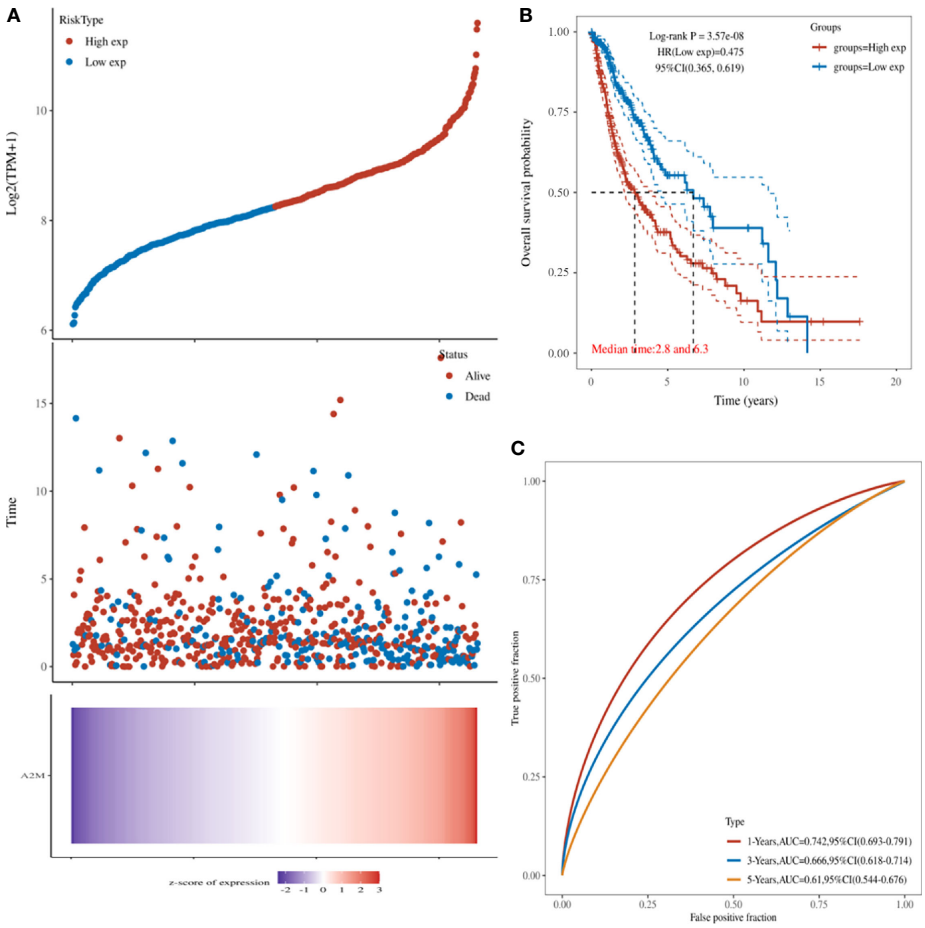

FIGURE 3 | POLD1 predicts survival of HCC patients. **(A)** HCC patients with high POLD1 expression show a significantly higher risk of death compared with those with low POLD1 expression. The z-score confirmed that high expression of POLD1 was associated with a higher mortality. **(B)** Kaplan–Meier curve demonstrated that high POLD1 expression was significantly correlated with worse OS; the median survival was 2.8 years in the POLD^{high} group and 6.3 years in the POLD^{low} group (n = 371 total patients). **(C)** ROC curve demonstrated the high sensitivity and specificity of the diagnostic and prognostic value of POLD1 expression.

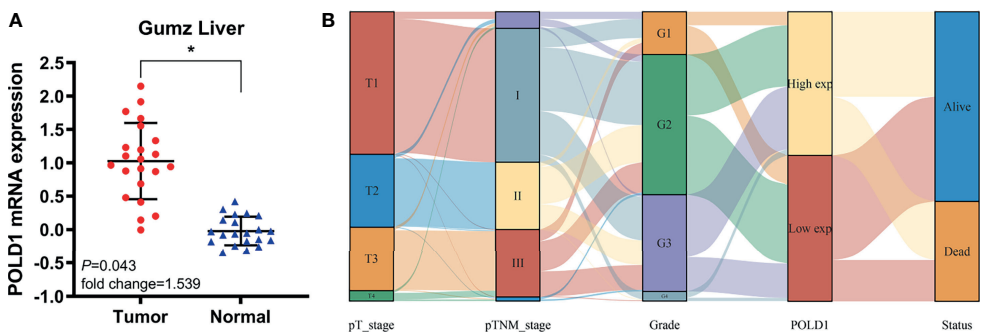

FIGURE 4 | POLD1 predicts aggressive clinic-pathologic parameters in HCC patients. **(A)** Significantly elevated POLD1 expression level was observed in HCC samples compared with normal samples in the Gumz Liver cohort (n = 22; *P* = 0.043). *p < 0.05. **(B)** The Sankey diagram shows the relationship between tumor grades, stages, POLD1 expression level and survival. Low tumor grade and early stage HCC patients tended to have lower expression of POLD1, while high tumor grade and advanced stage were related to the high expression of POLD1.

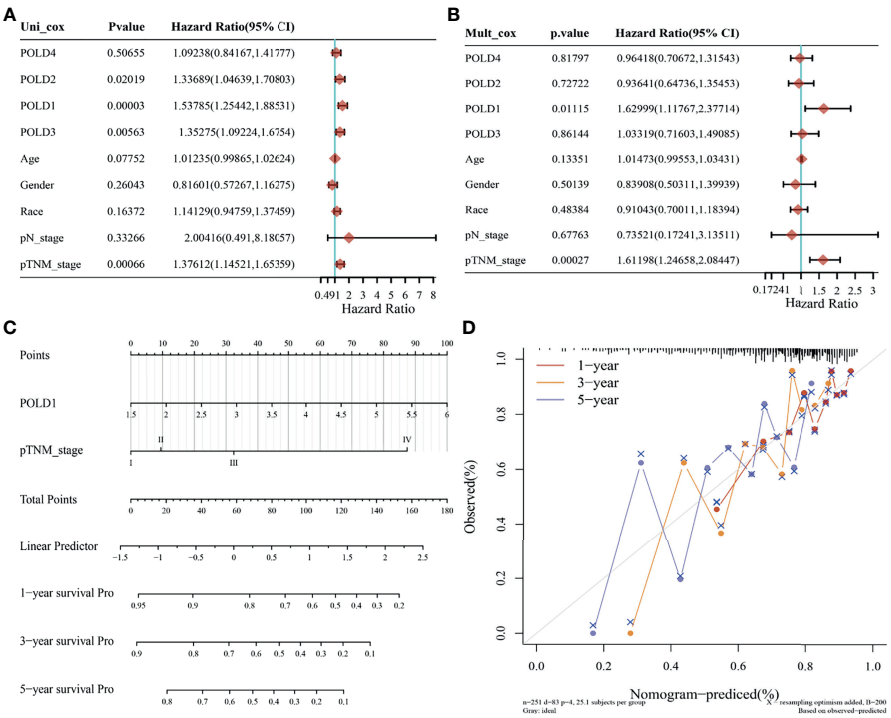

FIGURE 5 | Prediction model based on Cox regression and nomogram analysis. **(A)** Univariate Cox regression analysis indicated that POLD1, POLD2, POLD3 and pTNM stage were closely related to the survival of HCC patients ($P < 0.05$). **(B)** Multivariate Cox regression revealed a significant effect of POLD1 expression (HR = 1.63, $P = 0.011$) and pTNM stage (HR = 1.61, $P < 0.01$) on the prognosis of HCC. **(C)** We used a nomogram to evaluate the prognosis of HCC with a prediction model of POLD1 expression and pTNM stage. **(D)** a graphical representation of the factors was provided by nomogram to calculate the risk of recurrence for an individual patient. The prediction is more accurate in short-term survival prediction (C-index = 0.682, $P < 0.001$).

survival of HCC patients (P<0.05). Because of the interaction between these factors, multivariate Cox regression revealed a significant effect of POLD1 expression (HR=1.63, P=0.011) and pTNM stage (HR=1.61, P<0.01) on the prognosis of HCC (**Figures 5A, B**). Together, our findings have demonstrated a consistent relationship between high POLD1 expression and advanced clinicopathological staging. Thus, we included POLD1 expression and pTNM stages in a prediction model to evaluate the prognosis of HCC using a nomogram (**Figure 5C**). Finally, the nomogram provided a graphical representation of the factors, which was used to calculate the risk of recurrence for an individual patient based on the points associated with each risk factor, and the model was more accurate in short-term survival prediction (C-index=0.682, P<0.001; **Figure 5D**).

Univariate Cox regression analysis indicated that POLD1, pTNM staging and grade were closely related to the survival of HCC patients (P<0.05), and multivariate Cox regression revealed the significant effects of POLD1 expression (HR=1.54, P=0.0005) and pTNM staging (HR=1.27, P=0.016) on the prognosis of HCC (**Figures S3A, B**). These findings further confirm the relationship between high POLD1 expression and advanced clinicopathological staging in HCC prognosis. Therefore, we included POLD1 expression and pTNM stage in a nomogram to assess the prognosis of HCC patients (**Figure S3C**). The model is more accurate in short-term survival prediction (**Figure S3D**).

Landscape of POLD1 Mutation and Related Genes in HCC

In addition, to investigate the underlying role of POLD1, we explored the potential value of POLD1 mutation and other related genes based on multi-omics data. First, we found that the mutation was mainly due to POLD1 missense mutation on POLBc_delta and zf-C4pol (**Figure 6A**). However, the mutation frequency was only 1.1% in the TCGA cohort. Second, we analyzed the frequency of mutations in common genes in HCC according to differential POLD1 expression in 272 cases, including TP53, TTN, CTNNB1, MUC16, ALB, PCLO, RYR2, MUC4, ABCA13, APOB and POLD1. The highest mutation frequency was found in TP53 (28%). Interestingly, the $POLD1^{high}$ group exhibited significantly more TP53 mutations compared with the $POLD1^{low}$ group, suggesting the importance of the TP53 expression level in clinical treatment and tumor prognosis (**Figure 6B**). Then, we explored significant DEGs between the POLD1 altered and unaltered groups and found that DNAH5, TTN, TP53, NTAN1, HDAC5, TMEM51, KIAA1211, LMBR1, MCF2L and OR5L1 were markedly upregulated, whereas TP53 expression was significantly

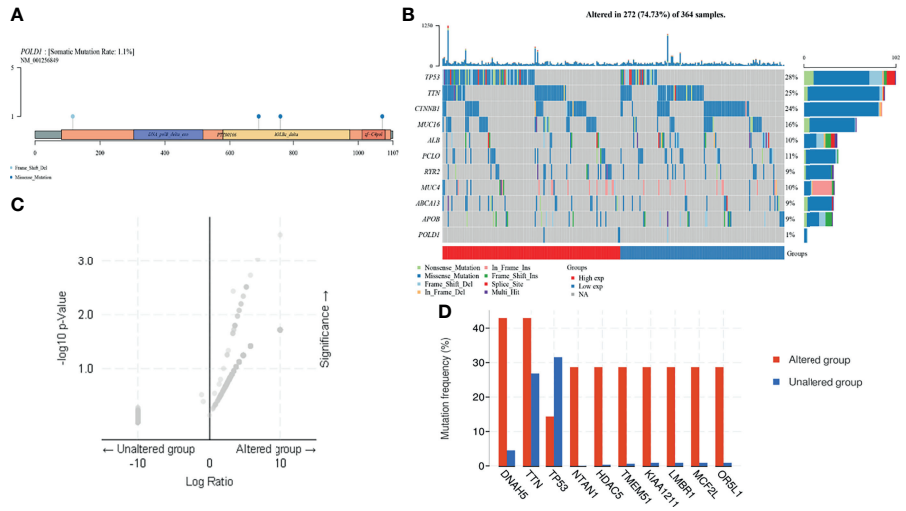

FIGURE 6 | POLD1 mutation analysis in HCC. **(A)** Mutation in POLD1 mainly occurs in POLBc_delta and zf-C4pol. **(B)** Mutations in genes that are commonly mutated in HCC, including *TP53*, *TTN*, *CTNNB1*, *MUC16*, *ALB*, *PCLO*, *RYR2*, *MUC4*, *ABCA13*, *APOB* and *POLD1*, were explored in 272 cases classified based on POLD1 expression. The highest mutation frequency was found in *TP53* (28%) and *TP53* mutation occurred more frequently in the POLD1^{high} group than the POLD1^{low} group. **(C–D)** Significant differentially expressed genes between POLD1 alteration and unaltered groups were identified and included up-regulated genes such as *DNAH5*, *TTN*, *TP53*, *NTAN1*, *HDAC5*, *TMEM51*, *KIAA1211*, *LMBR1*, *MCF2L* and *OR5L1* and the down-regulated gene *TP53*.

decreased in the altered group compared with the unaltered group (**Figures 6C, D**).

PPI Network Establishment and Functional Enrichment Analysis

To explore the mechanism of POLD1 in the DDR pathway, we constructed a PPI network and identified a gene panel of critical genes, including POLD1, POLD2, POLD3, POLD4, PCNA, MSH2, MSH6, RPA1, RPA3 and LIG1 (**Figure 7A**). Additionally, we studied the correlation between these genes. Except for POLD4, the other ten hub genes showed a close linear association with each other (Spearman's test; **Figure 7B**). To analyze the function of these genes, we conducted pathway enrichment analyses, including GO (BP, CC and MF), KEGG and Reactome (**Figures 7C, D**). We identified several critical factors, such as mismatch repair and DNA replication in BP, replication fork and a protein–DNA complex in CC, damaged DNA binding and nucleotidyltransferase activity in MF, nucleotide excision repair and base excision repair in KEGG and extension of telomeres in Reactome.

POLD1 Expression Predicts the Abundance of Immune Cells and Immune Checkpoint Molecules in the HCC Microenvironment

High expression levels of POLD1 were found to be closely associated with tumor purity, immune scores and stromal scores in pan-cancers (**Figure 8A**). In addition, several immune cells, including B cells, T cells, dendritic cells, macrophages and neutrophils, were significantly associated with POLD1 expression to varying degrees in different cancers, especially HCC (r^2>0.4, **Figure 8B**). Moreover, POLD1 expression was related to the abundance of neutrophils and CD56 natural killer cells in HCC (**Figure 8C**). Meanwhile, POLD1 levels exhibited a strong relationship with most immune checkpoint molecules, including CD274, CD80, CD86, CTLA4, PDCD1 and TCGIT (**Figure 8D**). The scatter plot shown in **Figure 8** demonstrates the close relationship between POLD1 expression levels and tumor purity (HR=0.141), B cells (HR=0.468), $CD8^+$ T cells (HR=0.277), $CD4^+$ T cells (HR=0.358), macrophages (HR=0.397), neutrophils (HR=0.364) and dendritic cells (HR=0.438). Overall, these findings suggested that POLD1 expression was correlated with the infiltration of several immune cells and reshaped the immune-excluded microenvironment.

Validation of Differential POLD1 Expression and Its Prognostic Value in the AHYMUN Cohort

To validate the increased expression of POLD1 in HCC samples compared with normal liver tissues, we first collected samples from HPA and explored the prognostic implications of POLD1 expression in 107 HCC patients from the AHYMUN cohort. The nuclear expression of POLD1 was significantly higher in HCC than adjacent normal tissues based on the HPA database and AHYMUN cohorts (**Figures 9A, B**). Additionally, the results suggested that increased protein expression of POLD1 was closely associated with worse OS (*P*=0.018, HR=1.697) and PFS (*P*=0.042, HR=1.669; **Figures 9C, D**).

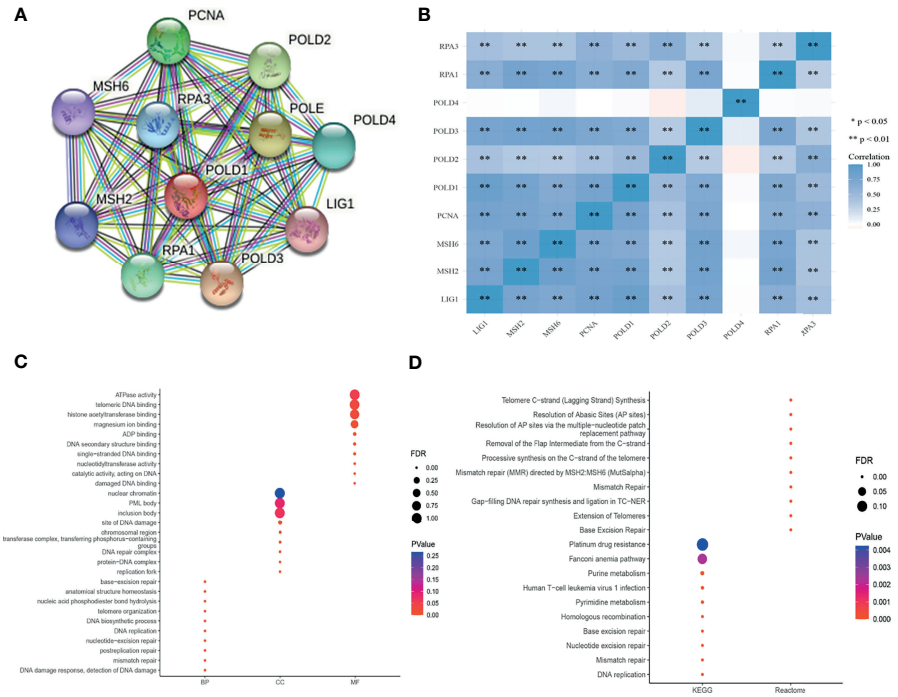

FIGURE 7 | PPI network establishment and functional enrichment analysis. **(A)** A protein–protein interaction network_ was constructed and hub proteins were identified, including POLD1, POLD2, POLD3, POLD4, PCNA, MSH2, MSH6, RPA1, RPA3 and LIG1. **(B)** Except for POLD4, the other ten hub genes showed strong correlations with each other in Pearson's test. **p < 0.01. **(C–D)** GO function annotation, KEGG pathway and Reactome pathway enrichment analyses were used to analyze the hub genes. The results identified mismatch repair and DNA replication in the biological process, replication fork and protein–DNA complex in cellular components, damaged DNA binding and nucleotidyltransferase activity in molecular function, nucleotide excision repair and base excision repair in KEGG, and extension of telomeres in Reactome analyses.

DISCUSSION

HCC is the most common primary liver cancer and has a poor prognosis. Over the past few decades, the incidences of liver cancer and liver cancer-related deaths have increased (4, 22). However, the treatment options for advanced liver cancer are very limited, and strategies for advanced personalized treatment of HCC are lacking (23). Therefore, better understanding of the mechanisms underlying HCC is critical because these findings may help identify novel treatments for HCC patients. In this study, we examined the potential prognostic value of POLD in HCC and evaluated its role in the tumor immune microenvironment.

POLD1 interacts with POLD2, POLD3, and POLD4 to form the POLδ holoenzyme together with replication factor C and proliferating cell nuclear antigen. POLD2–4 are smaller subunits, and POLD1 plays a major role in the biochemical activity of the polymerase (24). Consistent with these data, we found that POLD1 showed a more significant influence on tumor prognosis than the other three POLD proteins and therefore we focused on POLD1 for the subsequent analysis.

POLδ has important proofreading capabilities conferred by exonuclease activity and is also involved in repairing DNA damage, including nucleotide excision repair, double-strand break repair, base excision repair, and mismatch repair (25, 26). Multiple studies have linked germline and sporadic mutations in POLD1 and other subunits of POLδ with human pathologies (27, 28). For example, mutations in POLδ in mice and humans lead to genome instability, mutant phenotypes, and tumorigenesis. In addition, mutations in the proofreading domain of POLD1 have been identified as the root cause of some hereditary cancers and these mutations may affect treatment management. Recent studies have shown that loss-of-function alterations in DDR genes are associated with human tumorigenesis (29). Germline mutations in POLE and POLD1 have been shown to predispose patients to multiple colorectal polyps and a wide range of neoplasms (30). A previous study showed that at least 1 in 92 primary liver cancer patients had DDR gene mutation (31). The landscape of DDR mutations and their association with genetic and clinicopathologic features suggest that PLC patients with altered DDR genes may be rational candidates for precision treatment (32). Therefore, in addition to the increased expression level of POLD1, POLD1 and POLE mutations may function as independent biomarkers to predict the benefit of targeted treatment.

The expression levels of POLD family members in tumor and normal tissues were explored. According to previous research, the increased expression of POLD3 indicated a poor prognosis of HCC patients, although not as significantly as POLD1 (33).

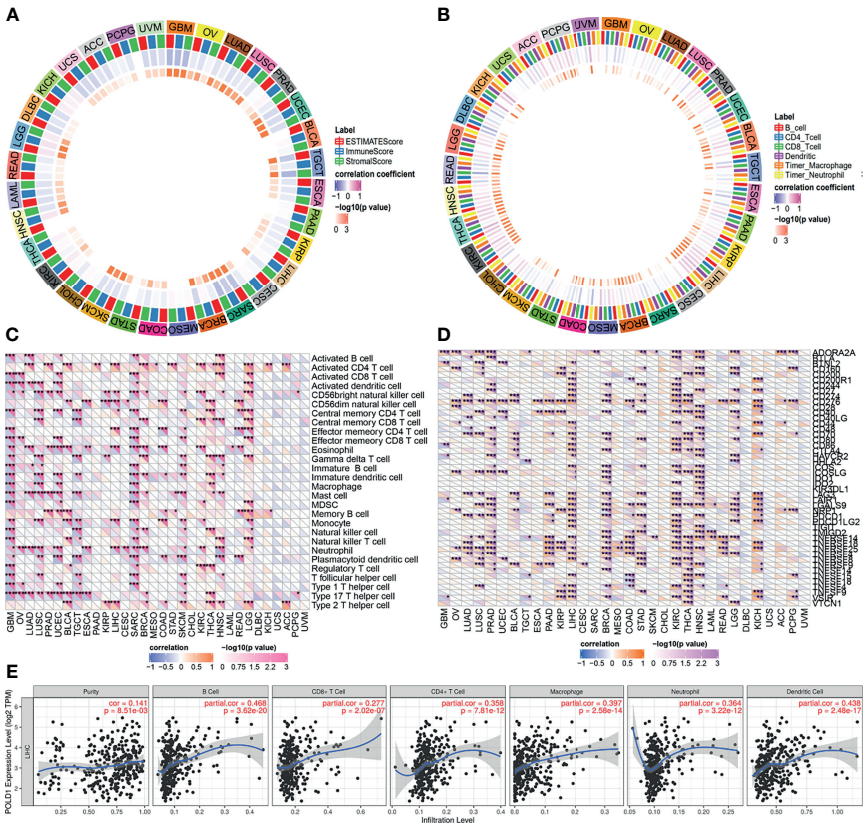

FIGURE 8 | Correlation between POLD1 expression and immune cells. **(A)** High expression of POLD1 was closely associated with tumor purity, immune score and stromal score in pan-cancers. **(B)** Immune cells, including B cells, T cells, dendritic cells, macrophages and neutrophils, were significantly associated with POLD1 expression in many cancers in varying degrees, especially in HCC. **(C–D)** POLD1 was related to the abundance of neutrophils and CD56 natural killer cells in HCC, and POLD1 expression showed strong relationships with most immune checkpoint molecules, including CD274, CD80, CD86, CTLA4, PDCD1 and TCGIT. *p < 0.05, **p < 0.01, ***p < 0.001. **(E)** A scatter plot shows the close relationship between POLD1 expression level and tumor purity (HR=0.141), B cells (HR=0.468), $CD8^+$ T cells (HR=0.277), $CD4^+$ T cells (HR=0.358), macrophages (HR=0.397), neutrophils (HR=0.364) and dendritic cells (HR=0.438).

POLD3 plays a specialized role in the repair of damaged replication forks, indicating that POLD3 activity may be particularly relevant for cancer cells enduring high levels of DNA replication stress (12). The cellular depletion of POLD1 or POLD3 resulted in differential genome instability manifested by DNA double-stranded breaks (17, 34).

Human cancers can be divided into three types according to the anti-tumor immune response status, or the immune phenotype: inflamed, immune-excluded, and immune desert status. Inflamed cancers generally refer to tumors with high PD-L1 expression in cancer cells and more immune cells and tumor infiltrating lymphocytes in the tumor; these tumors are sensitive to immune checkpoint inhibitors. Immune-excluded tumors are tumors in which the stroma shows a large number of T cells, but the T cells cannot penetrate the stroma and infiltrate the tumor because of the strong inhibitory microenvironment. These tumors often do not respond well to immune checkpoint inhibitors. Immune desert tumors lack the infiltration of T cells and immune cells even in the interstitium, which is a described as an "immune desert". In our analysis, we found that POLD1 is closely related to the expression of B cells, CD8+ T cells, CD4+ T cells and macrophages. These results suggest that POLD1 may play an important role in the immune-excluded tumor microenvironment. We speculate that this may be because POLD1 is involved in DNA cleavage repair, which affects the tumor microenvironment.

There are some advantages of this study. First, this research contained independent HCC cohorts, including the TCGA database (n=423), GTEx database and Gumz Renal cohort (n=10), and the real-world AHYMUN cohort (n=107). Second, we demonstrated the value of significantly elevated POLD expression for HCC prognosis and identified POLD1 as the most valuable gene for further analysis. Third, at both the mRNA and protein levels, we validated the association between POLD1 expression and HCC prognosis. Moreover, ~12,000 tumor samples from TCGA database were collected, and the effect of POLD1 expression on prognosis was verified in pan-cancers. Finally, functional enrichment analysis was performed. The role

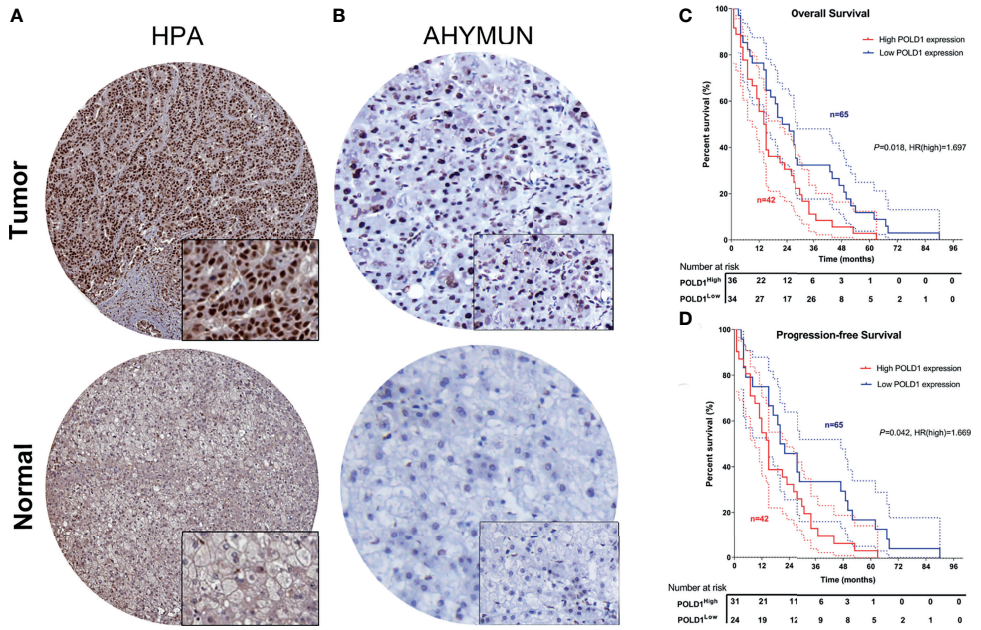

FIGURE 9 | Validation of differential POLD1 expression and its prognostic value in the AHYMUN cohort. **(A, B)** IHC analysis of samples from the AHYMUN cohort showed that the expression of the POLD1 protein was significantly higher in HCC than in normal liver tissues. **(C, D)** Prognostic implications of POLD1 expression in 107 HCC patients from the AHYMUN cohort indicated that a high POLD1 protein level was significantly associated with worse OS (P = 0.018, HR = 1.697) and PFS (P = 0.042, HR = 1.669).

of POLD1 in the infiltration of immune cells in the tumor microenvironment was demonstrated, which may guide cancer treatment and targeted drug development.

This study has several limitations. First, there is a high degree of heterogeneity between the patient groups in this study. Therefore, in the next study, we will select patients from multiple regions for multi-center research. Second, this was a retrospective study. Finally, we will further study the demographic, clinical and pathological details of the population.

Our study has demonstrated a link between elevated POLD1 expression and patient survival and the tumor microenvironment in HCC. In the next study, we will explore the role and mechanism of POLD in HCC progression through cytological tests and animal experiments. We will also analyze the potential mechanisms of POLD, POLδ, and DNA cleavage repair in HCC through high-throughput sequencing and other methods. These findings will help provide new insights into the pathogenesis of HCC and new ideas for the personalized treatment of HCC patients.

CONCLUSION

This study first investigated the molecular and clinical role of the POLD family and revealed the significant relationship between elevated POLD1 expression and the poor survival and immune-excluded tumor microenvironment of HCC patients. Together, these findings support the use of POLD1 as a biomarker to guide the molecular diagnosis and development of novel targeted therapeutic strategies for HCC 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 study design and experimental procedures were performed in accordance with the Declaration of Helsinki. The study was approved by the ethics committee of the Affiliated Hospital of Youjiang Medical College for Nationalities (Baise, Guangxi Province, China). Written informed consent was obtained from all participants.

AUTHOR CONTRIBUTIONS

WL and SZ carried out the molecular genetic studies, participated in the sequence alignment and drafted the

manuscript. JW and SC carried out the immunoassays. HD, CW and BH participated in the sequence alignment. XS and QG participated in the design of the study and performed the statistical analyses. HT, SZ, YZ, WL, and JW conceived the study, participated in the study design and coordination and helped to draft the manuscript. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2021.736363/full#supplementary-material

Supplementary Figure 1 | Flowchart of the study.

Supplementary Figure 2 | High expression of POLD1 in pan-cancers compared with normal liver tissues. **(A, B)** POLD1 expression levels between pan-cancers and normal tissues were compared in the TCGA (n = 423) and GTEx (n = 533) datasets. POLD1 expression was differentially expressed in cancer tissue and adjacent normal tissue in pan-cancer, and the expression of POLD1 was significantly higher in the HCC tumor group compared with the normal group. **(C, D)** The POLD expression level in pan-cancers and normal tissue based on data from the TCGA and GTEx databases. The results are unremarkable in some cancers. **(C, D)** Cox regression analysis showed that POLD1 expression is closely correlated with OS in many cancers.

Supplementary Figure 3 | Prediction model based on Cox regression and nomogram analysis of POLD1. **(A)** Univariate Cox regression analysis suggested that POLD1, age, grade, race and pTNM stage were closely related to the survival of HCC patients ($P < 0.05$). **(B)** Multivariate Cox regression revealed the significant effect of POLD1 expression and pTNM stage on the prognosis of HCC. **(C)** We used a nomogram to evaluate the prognosis of HCC with a prediction model of POLD1 expression and pTNM stage. **(D)** A graphical representation of the factors was provided by nomogram to calculate the risk of recurrence for an individual patient. The prediction is more accurate in short-term survival prediction.

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Surgical Conversion for Initially Unresectable Locally Advanced Hepatocellular Carcinoma Using a Triple Combination of Angiogenesis Inhibitors, Anti-PD-1 Antibodies, and Hepatic Arterial Infusion Chemotherapy: A Retrospective Study

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Background: Recent research has shown that selected patients with initially unresectable hepatocellular carcinoma (HCC) are able to achieve conversion to resectable disease through systemic or local therapy. Combination regimens comprised of drugs with different mechanisms of action have shown better outcomes than single-drug or single-approach-based treatments; however, to date, combination regimens investigated as part of conversion therapy strategies have been two drug combinations with reported issues of relatively low surgical conversion and objective response rates. In this study, we investigated the efficacy and safety of triple combination therapy with angiogenesis inhibitors, programmed death-1 inhibitors and hepatic arterial infusion chemotherapy for surgical conversion of advanced HCC.

Methods: This was a single-center, retrospective, single-arm study of patients with unresectable HCC who received at least one cycle of triple combination therapy with an oral anti-angiogenic drug, programmed death-1 inhibitors and hepatic arterial infusion chemotherapy between August 2019 and August 2020. Endpoints included the overall response rate (ORR), surgical conversion rate, time to response and safety. Treatment response was assessed using the modified Response Evaluation Criteria in Solid Tumors (mRECIST) and RECIST v1.1.

Results: In total, 34 patients were included in this study, of whom 25 completed treatment evaluation. The best ORR was 96.0% (24/25); 48.0% (n = 12) had a

complete response, 48.0% (n = 12) had a partial response, and 4.0% (n = 1) had stable disease. The median time to response was 50.5 (95% CI, 31.02–64.00) days and the surgical conversion rate was 60% (15/25). Of the 25 patients, 56.0% (n = 14) received surgical resection and 28.0% (n = 7) had a pathologic complete response. Toxic side effects were manageable.

Conclusion: A triple combination therapy regimen of angiogenesis inhibitors, programmed death-1 inhibitors and hepatic arterial infusion chemotherapy showed significant therapeutic effect with an extremely high surgical conversion rate in patients with initially unresectable HCC.

Keywords: advanced hepatocellular carcinoma, combination therapy, hepatectomy, conversion therapy, hepatic arterial infusion chemotherapy, anti-PD-1, China

INTRODUCTION

Hepatocellular carcinoma (HCC) is the sixth-most-common cancer and the third leading cause of cancer-related deaths worldwide (1). Owing to the absence of symptoms in the early stages of the disease, more than 70% of patients with HCC are diagnosed at an advanced stage, long after transplantation, surgery or locoregional treatment are feasible (2). Palliative systemic therapy is usually the only remaining treatment option for these patients. However, in the past few years, treatment of advanced HCC has evolved rapidly with the introduction of novel systemic therapies.

Although the mechanisms of action of new therapies for HCC are not fully understood, therapeutic regimens that combine drugs with different mechanisms of action have shown significantly better outcomes than single-drug or single-approach-based treatments. Combination regimens that have been investigated include two-drug combinations such as two immune checkpoint inhibitors, immune checkpoint inhibitors with molecular-targeted drugs or sequential use of two molecularly-targeted drugs (3–10); immune checkpoint inhibitors with locoregional treatment (4, 11); and molecularly-targeted drugs with locoregional treatment (12–14). The furthest advanced combination therapy to date is dual combination treatment with a tyrosine kinase inhibitor and immune checkpoint blockade (atezolizumab plus bevacizumab) which is a recommended first-line therapy for advanced HCC based on the phase 3 IMbrave150 trial in which it showed a significant survival benefit versus sorafenib (8).

In previous studies, hepatic arterial infusion chemotherapy (HAIC) demonstrated relatively high response rates (22–86%) and an acceptable toxicity profile (15, 16). In Japan, HAIC is considered a safe and effective alternative to sorafenib and is recommend for use in patients with advanced HCC (17). Recent clinical trials have also shown that sorafenib plus HAIC with oxaliplatin, fluorouracil, and leucovorin (FOLFOX) improved the objective response rate (ORR) versus sorafenib in patients with HCC and portal vein invasion; however, the survival benefit was unsatisfactory (18). Immune combination therapy is associated with the 'survival drag effect' in which patients with an effective immune response will achieve a highly durable antitumor response from a particular time point (the onset of the immune response) (19). In summary, the ORR of HAIC is high, but does not translate to an increase in OS. This can be compensated through use of a dual combination of a tyrosine kinase inhibitor and immune checkpoint blockade.

In clinical practice, selected patients with unresectable HCC can be converted to resectable disease through a variety of systemic or locoregional treatment strategies, and some studies have shown that salvage surgery following surgical conversion can achieve favorable outcomes in these patients. An effective way to improve OS with combination therapy is to proactively conduct radical treatments, such as conversion surgery or ablation (20). Indeed, salvage surgery following surgical conversion has been reported to achieve favorable outcomes in some studies (21). However, challenges reported with previously investigated approaches include relatively low objective response rates (ORRs) and surgical conversion rates, highlighting a need for the identification of more effective combination regimens with manageable side effects in order to allow a higher proportion of patients to achieve conversion to resectable disease. The present study investigated the efficacy and safety of a triple combination therapy strategy including angiogenesis inhibitors, programmed death-1 (PD-1) inhibitors and HAIC in patients with initially unresectable advanced HCC.

MATERIALS AND METHODS

Study Design and Patients

This single-center, retrospective, single-arm study included patients aged ≥18 years with unresectable HCC confirmed by three independent hepatobiliary surgeons, with one or more measurable target lesions based on computerized tomography (CT) or magnetic resonance imaging (MRI) who had received triple therapy at Tianjin Medical University Cancer Institute and Hospital between August 1, 2019 and August 20, 2020. Eligible patients had not received previous treatment for HCC or had progressed on previous treatments, and had Barcelona Clinic Liver Cancer (BCLC) stage C disease, an A or B on the Child-Pugh liver function scale and an Eastern Cooperative Oncology Group Performance Status score of 0–2. Exclusion criteria

included comorbidity with other severe systemic diseases, discontinuation of treatment for personal reasons or violating treatment procedures, and inability to tolerate or comply with treatment.

The study protocol was approved by the The Research Ethics Committee of Tianjin Medical University Cancer Institute and Hospital, which granted ethical approval for the use of human subjects (Approval No. bc2020007). The study was conducted in accordance with the Declaration of Helsinki and other ethical principles for medical research involving human subjects. All patients gave written informed consent before entering the study.

Treatments

Patients were treated with a triple combination of angiogenesis inhibitors, anti-PD-1 antibodies, and HAIC. As long as a triple combination regimen was used, the brand of angiogenesis inhibitors and anti-PD-1 antibodies was not considered. Three kinds of angiogenesis inhibitors and two kinds of anti-PD-1 antibodies, which are commonly used in clinical practice, were used by patients in this study. To be more specific, eligible patients had received a triple combination of an oral anti-angiogenic drug (in order to reduce side effects and ensure curative effect, a low dose was used, based to the prescribing information: apatinib 250 mg/day, lenvatinib 8 mg/day, or sorafenib 400 mg twice daily), a PD-1 inhibitor (camrelizumab 200 mg or sintilimab 200 mg every 3 weeks) administered intravenously, and HAIC with FOLFOX administered every 4–8 weeks with the catheter and sheath removed at the end of infusion. The choice of anti-angiogenic agent was at the discretion of the patient. The FOLFOX regimen comprised oxaliplatin 85 mg/m^2 as a 2 hours infusion, calcium folinate 400 mg/m^2 as a 2–3 hours infusion and fluorouracil 400 mg/m^2 as a bolus injection, followed by fluorouracil 1200 mg/m^2 administered over 23 hours on day 1. HAIC dosing was adjusted appropriately according to the patient's liver function with a minimum dosage of one third of the standard dose. Imaging evaluations (MRI was preferred and CT was used if MRI was not available) were conducted every 4-8 weeks after at least one HAIC treatment. Tumors were assessed every 2 months using serum markers and imaging examination with abdominal ultrasound as the primary method and contrast MRI on suspicion of recurrence. Patients determined to have sufficient residual liver volume after tumor resection, verified by senior surgeons during multidisciplinary meetings, received surgical intervention followed by oral angiogenesis inhibitors and PD-1 inhibitors.

Measurements and Endpoints

Endpoints included ORR, defined as the percentage of patients with complete response (CR) or partial response (PR), time to response (TTR), progression-free survival (PFS), surgical conversion rate, 6-month PFS, 12-month PFS and safety. Safety was assessed according to the National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE). Tumor response was assessed using the modified Response Evaluation Criteria in Solid Tumors (mRECIST) and RECIST v1.1 (22, 23).

Statistical Analyses

Safety and efficacy analyses were conducted for all patients who received at least one cycle of triple combination therapy and completed clinical evaluation. For baseline characteristics, variables were expressed as frequencies and percentages, and data were expressed as either median (standard deviation) or mean (range). Survival data were analyzed using the Kaplan–Meier method. OS was calculated from the date of initiation of triple combination therapy until death or last follow-up. PFS was calculated from the date of initiation of triple combination therapy until disease progression, recurrence or last follow-up. All data were analyzed using SPSS version 23.

RESULTS

Patients

A total of 34 patients had received triple combination therapy, of whom four failed to complete the procedure because of gastrointestinal bleeding (two with grade 3 and two with grade 1), two did not complete the procedural requirement to perform imaging tests 4–8 weeks after the last HAIC treatment and three had a HAIC interval >8 weeks for personal reasons. Ultimately, 25 patients [19 males and six females; median age: 59 years (range: 49–78 years)] were included in the analysis (**Figure 1**). The follow-up ended on May 1, 2021.

All patients included in the analysis had HCC considered unresectable for three reasons: 1) the main portal vein or inferior vena cava were invaded (n = 10); 2) multiple lymph node metastases or intrahepatic lesions could not be radically resected (n = 6); 3) the residual liver volume was insufficient after radical resection (n = 9). Of these 25 patients 100% had BCLC stage C disease, 22 (88.0%) had Child–Pugh grade A liver function and three (12.0%) had Child–Pugh grade B, 22 (88.0%) had hepatitis B and two (8.0%) had hepatitis C, 20 (80.0%) had liver cirrhosis, six (24.0%) had esophago-gastric varices and 23 (92.0%) had elevated alpha-fetoprotein (AFP) levels (AFP >7 ng/mL), including 10 (40.0%) with AFP >1000 ng/mL. At baseline, according to the Japanese grading system for tumor emboli (24), there were 18 patients with portal vein invasion (Vp2, Vp3, and Vp4 grades: four, seven, and seven patients, respectively), four patients with hepatic vein invasion (Vv2 and Vv3: two and two patients, respectively), and one patient with both invasion of the portal vein and the inferior vena cava (Vp3 and Vv3, one patient). In addition, ten patients with an invasion of the main trunk of the portal vein (Vp4, n = 7) or inferior vena cava (Vv3, n = 3) were defined as "super-advanced" patients. The number of patients with multiple intrahepatic foci and extrahepatic lymph node metastases, confirmed by imaging, was six (24.0%) and 12 (48.0%), respectively (**Table 1**).

Treatment

For angiogenesis inhibitor, patients chose apatinib (six patients, 125–250 mg/day, mean 225 mg/day), lenvatinib (18 patients, 4–8 mg/day, mean 6.87 mg/day), or sorafenib (one patient, 400 mg BID), based mainly on their economic situation. All patients also

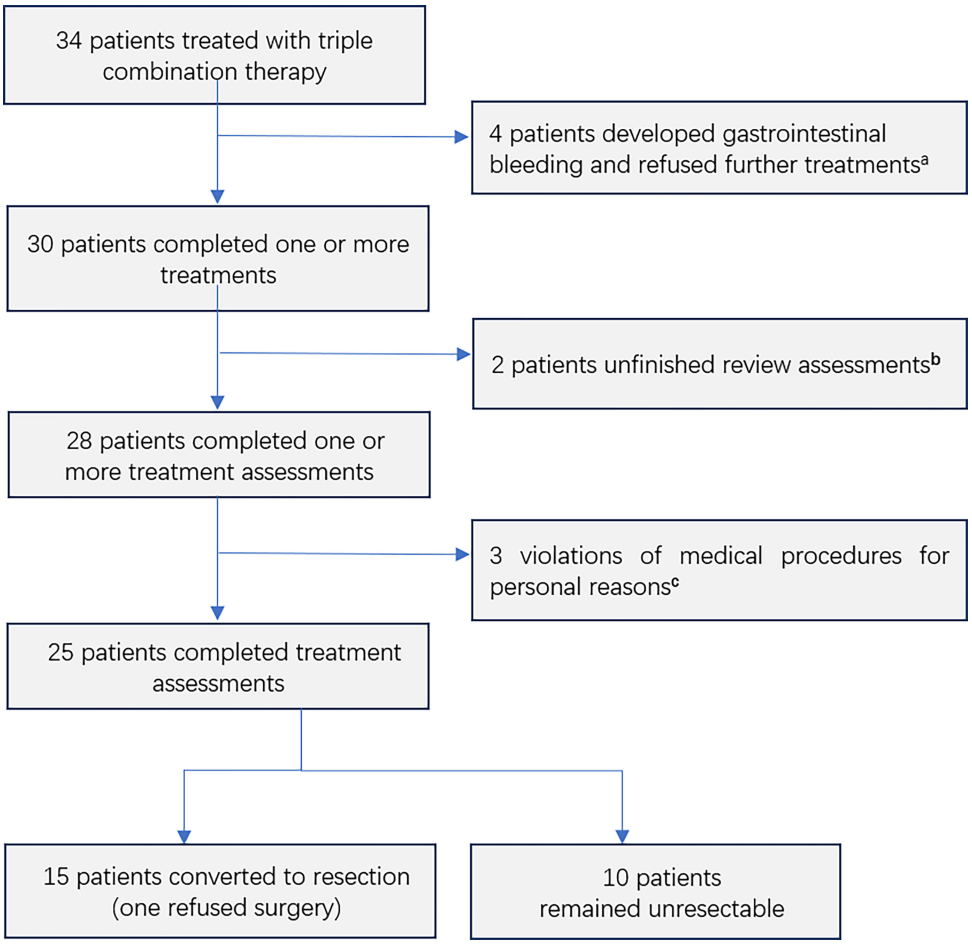

FIGURE 1 | Consolidated Standards of Reporting Trials diagram including all 34 patients who entered into the study. [a]All four patients had gastric fundus varices owing to portal hypertension. Two patients had grade 3 gastrointestinal bleeding, which was treated endoscopically. Two patients had grade 1 gastrointestinal bleeding, which was not treated. [b]Two patients did not follow the procedure requiring a review of imaging 4–8 weeks after the last HAIC treatment. [c]The interval between the two HAIC treatments was >8 weeks for personal reasons.

received anti-PD-1 monoclonal antibodies intravenously every 3 weeks (13 patients received camrelizumab and 12 sintilimab). In general, five different drug regimens were used, including sorafenib with camrelizumab (n = 1), apatinib with camrelizumab (n = 5), lenvatinib with camrelizumab (n = 7), apatinib with sintilimab (n = 2) and lenvatinib with sintilimab (n = 10). The mean HAIC interval was 45.9 days, the mean number of doses received was 2.96 (patients who achieved conversion to surgical treatment received fewer courses of HAIC than the non-converted group: 2.47 *vs.* 4.13) and the mean dose of the component therapies received was: oxaliplatin, 72.9 mg/m^2; calcium folinate, 269.2 mg/m^2; fluorouracil bolus, 341.2 mg/m^2; and fluorouracil infusion, 971.4 mg/m^2.

Safety

Most patients (92.0%, 23/25) experienced adverse events with varying severity (**Table 2** and **Supplementary Table 1**), with 84.0%, 68.0%, 28.0%, and 0% of patients experiencing grade 1, 2, 3 and 4-related adverse events, respectively. The most common treatment-related adverse events of any grade were neutropenia (36.0%, n = 9), leukopenia (32.0%, n = 8), elevated aspartate aminotransferase levels (28.0%, n = 7), anemia (28.0%, n = 7), elevated alanine aminotransferase levels (24.0%, n = 6), and hypoproteinemia (24.0%, n = 6). Treatment-related adverse events resulted in three lenvatinib treatment dose reductions (17.6% of all lenvatinib patients) and one apatinib treatment dose reduction (14.2% of all apatinib patients). Treatment-related adverse events resulted in reduced doses of HAIC in 10 (40.0%) patients. Two (8.0%) patients received endoscopic hemostasis for gastrointestinal bleeding (grade 3) that delayed HAIC treatment.

Tumor Response

Treatment efficacy was evaluated based on the investigator's assessment using mRECIST and RECIST v1.1. However, for consistency, only assessments using mRECIST are summarized

TABLE 1 | Baseline demographic and clinical characteristics (n = 25).

Characteristics	No.
Age, years	
Median	61.95
Range	49–78
Gender	
Male	19
Female	6
Hepatitis B virus infection	22
Hepatitis C virus infection	2
Child-Pugh classification	
A	22
B	3
BCLC stage	
B	0
C	25
Serum AFP level, ng/mL	
<400	11
400–1,000	4
≥1,000	10
Liver cirrhosis	20
Esophago-gastric varices	6
Macroscopic vascular invasion[a]	
Vp2	4
Vp3	7
Vp4	7
Vv2	2
Vv3	2
Vp3 & Vv3	1
Lymphatic metastasis	12
Intrahepatic metastasis	6

[a]*Vp2, invasion of (or tumor thrombus in) second order branches of the portal vein; Vp3, invasion of (or tumor thrombus in) first order branches of the portal vein; Vp4, invasion of (or tumor thrombus in) the main trunk of the portal vein and/or contra-lateral portal vein branch to the primarily involved lobe; Vv2, invasion of (or tumor thrombus in) the right, middle, or left hepatic vein, the inferior right hepatic vein, or the short hepatic vein; Vv3, invasion of (or tumor thrombus in) the inferior vena cava (24).*

BCLC, Barcelona Clinic Liver Cancer; PVTT, portal vein tumor thrombus; IVCTT, inferior vena cava tumor thrombus.

in the following section. The best ORR was 96.0% and the median TTR was 50.5 days (95% CI: 31.02–64.00) (**Table 3**). A CR was observed in 12 patients (48.0%), a PR in 12 patients (48.0%), and stable disease (SD) in one patient (4.0%). The efficacy of the different drug combinations was summarized and all showed satisfactory results (**Supplementary Table 3**). During the follow-up period, three patients progressed from a PR to PD and eventually died. One of them progressed during treatment and had a time-to-progression (TTP) of 182 days. The other two discontinued interventional treatment after two cycles of HAIC owing to the COVID-19 pandemic.

Among the nine patients who could not complete treatment, seven had an imaging review; two of these achieved PR, one had PD and four had SD. The last two patients had no imaging review and could not be evaluated for efficacy (see **Supplementary Table 2** for all 32 patients who were treated and had imaging evaluations).

AFP Response to Treatment

Before treatment, the median AFP level was 539.30 ng/mL (95% CI: 82.82–1310.00) and decreased to 10.20 ng/mL (95% CI: 4.64–28.32) after the first cycle of treatment. As of March 1, 2021, in 19 patients (82.6% of patients with elevated AFP at baseline) AFP levels had returned to the normal range (<7 ng/mL). The AFP level changes in all 25 patients are shown in **Figure 3E**.

Long-Term Outcomes

Fifteen patients (60.0%) met the surgical criteria for tumor and embolus regression; 14 (56.0%; one refused surgery) underwent surgical resection; and seven (28.0%) achieved a pathologic CR that was confirmed post-operatively. One patient had post-operative intrahepatic recurrence, whose relapse-free survival (RFS) was 13.17 months.

Of the 25 patients included in the analysis, 14 (56%) were followed up for more than 12 months (median follow-up time: 15.85 months [range: 12.30–20.67 months]), and their 6-month and 12-month PFS rates were 92.9% and 92.9%, respectively. The other 11 patients (44%) were followed up for less than 12 months [median follow-up time: 9.73 months (range: 5.10–11.83 months)] had a 6-month PFS rate of 72.70%. For all patients in the analysis, the median follow-up duration was 12.53 months (interquartile range, 9.85–16.95 months) and during this time the median PFS and median OS was not reached (**Figure 2**). The 6-month PFS rate for the ten non-converted patients was 70%. Among the seven patients who survived but did not achieve surgical conversion, three had tumor volume reduction but not sufficiently to undergo radical surgery, three had tumor volume reduction but lymph node metastasis did not completely disappear, and one patient had no obvious change in tumor volume. The changes in maximum tumor diameter after treatment are presented in **Figure 3**, and representative imaging data are presented in **Supplementary Figures 1** and **2**.

DISCUSSION

Various combinations of treatments have been investigated for achieving surgical conversion in patients with advanced HCC including transarterial chemoembolization, HAIC, immunotherapy, chemoradiotherapy, and systemic chemotherapy. However, the reported conversion rates are far from satisfactory at 15-20% (25). Recent studies have suggested that the combination of HAIC-based locoregional therapy with targeted therapy and immunotherapy is a promising multimodal approach for advanced HCC (20). In the present study, we demonstrated a high ORR (96.0%) achieved in a relatively short time (median TTR 50.5 days) compared with previous reports of combination regimens (ORR: 23.1–54.4%) with the triple combination of angiogenesis inhibitors, PD-1 inhibitors and HAIC with FOLFOX, as well as a relatively high surgical conversion rate (60.0%) (3, 6–9, 11, 14). Our study investigated the combined effects of drugs with different mechanisms without limiting the types or brands of angiogenesis inhibitors and PD-1inhibitors. However, all combination regimens investigated in this study showed similar positive results, indicating universal applicability of the triple combination therapy. Furthermore, the high pathologic CR rate suggests that triple combination therapy can provide greater survival benefits, which is supported by the favorable 6- and 12-month PFS. However,

TABLE 2 | Summary of the most common treatment-related adverse events in patients with advanced hepatocellular carcinoma receiving triple therapy (n = 25).

Preferred AE term, n (%)	Any grade	Grade 1	Grade 2	Grade 3
Neutropenia	9 (36.0)	3 (12.0)	6 (24.0)	0
Leukopenia	8 (32.0)	2 (8.0)	5 (20.0)	1 (4.0)
Anemia	7 (28.0)	4 (16.0)	3 (12.0)	0
AST level increased	7 (28.0)	2 (8.0)	3 (12.0)	2 (8.0)
ALT level increased	6 (24.0)	3 (12.0)	2 (8.0)	1 (4.0)
Hypoalbuminemia	6 (24.0)	6 (24.0)	0	0
Serum bilirubin increase	5 (20.0)	4 (16.0)	1 (4.0)	0
Rash	5 (20.0)	2 (8.0)	3 (12.0)	0
Hypertension	4 (16.0)	0	3 (12.0)	1 (4.0)
Hyperglycemia	4 (16.0)	4 (16.0)	0	0
Oulorrhagia	4 (16.0)	3 (12.0)	1 (4.0)	0
Fatigue	3 (12.0)	3 (12.0)	0	0
Proteinuria	3 (12.0)	0	3 (12.0)	0
Diarrhea	2 (8.0)	2 (8.0)	0	0
Nausea	2 (8.0)	2 (8.0)	0	0
Pruritus	2 (8.0)	1 (4.0)	1 (4.0)	0
Edema peripheral	2 (8.0)	2 (8.0)	0	0
Epistaxis	2 (8.0)	2 (8.0)	0	0
Decreased appetite	2 (8.0)	2 (8.0)	0	0
Gastrointestinal bleeding	2 (8.0)	0	0	2 (8.0)
Hypothyroidism	1 (4.0)	0	1 (4.0)	0
Weight decreased	1 (4.0)	1 (4.0)	0	0
Abdominal distention	1 (4.0)	1 (4.0)	0	0
Arthralgia	1 (4.0)	1 (4.0)	0	0
Gastrohelcoma	1 (4.0)	0	1 (4.0)	0

AE, adverse event; ALT, alanine aminotransferase; AST, aspartate transaminase.

evaluation of median overall survival requires an extended follow-up period and could not be evaluated in this analysis.

Ninety-two percent of the patients in this analysis had macrovascular invasion (n = 23), including 10 patients (40.0%) with invasion of the main trunk of the portal vein or inferior vena cava, which was an exclusion criteria in most previous clinical trials of treatments for HCC. Although the small sample size of this study may have led to biased results, the results showed that this group of "super-advanced" patients had an ORR of 100% following triple therapy and a surgical conversion rate of 50.0% (5/10), including four patients (40.0%) who achieved a pathologic CR (**Table 3**). This result needs further validation over a more extended follow-up period.

The triple therapy protocol described in this study rapidly reduced tumor load and attenuated tumor activity, manifested by a rapid decrease in AFP level and tumor volume (**Figure 3**), extensive tumor necrosis and inactivation of cancer thrombus regression (**Supplementary Figures 1, 2**). These manifestations can be observed in data from a typical patient who had esophago-gastric varices and then grade 3 gastrointestinal bleeding after first cycle of treatment. Although the endoscopic hemostasis delayed subsequent treatment and AFP level was elevated, the tumor and emboli of the left portal vein showed complete necrosis after the second cycle of triple combination therapy (**Figure 4**). This high level of anti-tumor activity led to 60% of patients included in the analysis, all of whom had initially unresectable tumors, achieving sufficient reductions in tumor load to meet the standard for radical resection. Every one of the 15 converted patients received no more than three courses of HAIC treatment (mean 2.37 courses), while the non-converted

TABLE 3 | Summary of efficacy outcomes (n = 25).

Variables, n (%)	Best overall response (mRECIST)	Best overall response (RECIST v1.1)	Overall response at data cut-off (mRECIST)	Beyond criteria[a] best response (mRECIST)	Under criteria[b] best response (mRECIST)
Complete response	12 (48.0)	2 (8.0)	12 (48.0)	4 (40)	8 (53.3)
Partial response	12 (48.0)	19 (76.0)	9 (36.0)	6 (60)	6 (40.0)
Stable disease	1 (4.0)	4 (16.0)	1 (4.0)	0 (0)	1 (6.7)
Progressive disease	0 (0)	0 (0)	3 (12.0)	0 (0)	0 (0)
Objective response rate	24 (96.0)	21 (84.0)	21 (84.0)	10 (100)	14 (93.3)
Received hepatic resection	14 (56.0)	14 (56.0)	14 (56.0)	5 (50)	9 (60.0)
Pathologic complete response	7 (28.0)	7 (28.0)	7 (28.0)	2 (20)	5 (33.3)

[a]*Beyond criteria included patients with vein tumor thrombus Vp4 or Vv3;*
[b]*Under criteria included patients without the above states.*

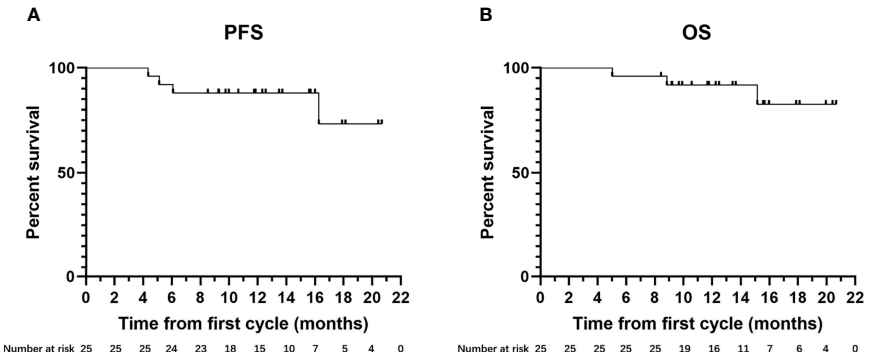

FIGURE 2 | Kaplan-Meier estimates of **(A)** progression-free survival (PFS) by modified RECIST (n = 25) and **(B)** overall survival (OS) (n = 25).

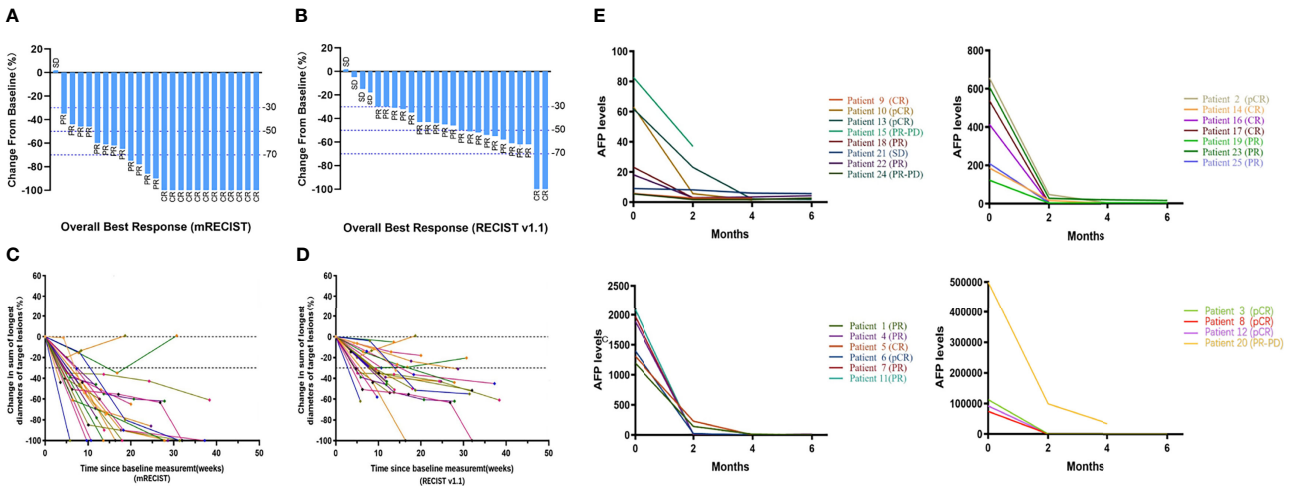

FIGURE 3 | Best percentage change in tumor burden and changes of AFP levels over time. Waterfall plot of best percentage change from baseline in sums of diameters of target lesions by **(A)** modified RECIST and **(B)** RECIST v1.1; Percentage change in target lesions diameters over time by **(C)** modified RECIST and **(D)** RECIST v1.1; **(E)** AFP levels of 25 patients before treatment, two months, four months and six months after first cycle of treatment, presented with their efficacy outcomes by modified RECIST, respectively.

patients received more courses (mean 4.13 courses), suggesting that rapid tumor response to treatment is associated with a high rate of conversion.

Surgical resection allowed patients to minimize tumor load before tumor progression due to drug resistance, thus increasing their chances of an effective cure and prolonged survival. Of the 14 patients who underwent surgery, nine (64.3%) had Vp3–4 portal vein tumor thrombosis (PVTT); after a median follow-up time of 18.13 months (range: 9.23–20.67months), only one post-operative intrahepatic recurrence was observed and treated with radiofrequency ablation. This is significant when compared with previous reports which have shown a median survival time of only 0.5–0.8 years in HCC patients with Vp3–4 PVTT (26). Furthermore, of the 14 patients who underwent resection, only the one patient who relapsed had an AFP level of >7 ng/mL after surgery, which suggests that AFP can be used as an indicator of postoperative recurrence. At the end of follow-up, the other 13 post-operative patients had no recurrence or metastasis on imaging assessment; however, median relapse-free survival could not be assessed.

The cohort of patients in this study had many characteristics usually associated with poor prognosis and limited treatment options, including a background of hepatitis [hepatitis B, n = 22 (88.0%); hepatitis C, n = 2 (8.0%)], vascular invasion [n = 23 (92.0%)], a baseline tumor diameter >10 cm [n = 13 (52.0%)], cirrhosis [n = 17 (68.0%)] and portal hypertension [n = 13 (52.0%)]. The liver function of the patients was also mostly in the compensatory stage affected by both cirrhosis and the tumor and was susceptible to deterioration by therapeutic agents. Therefore, in order to ensure safety and completion of

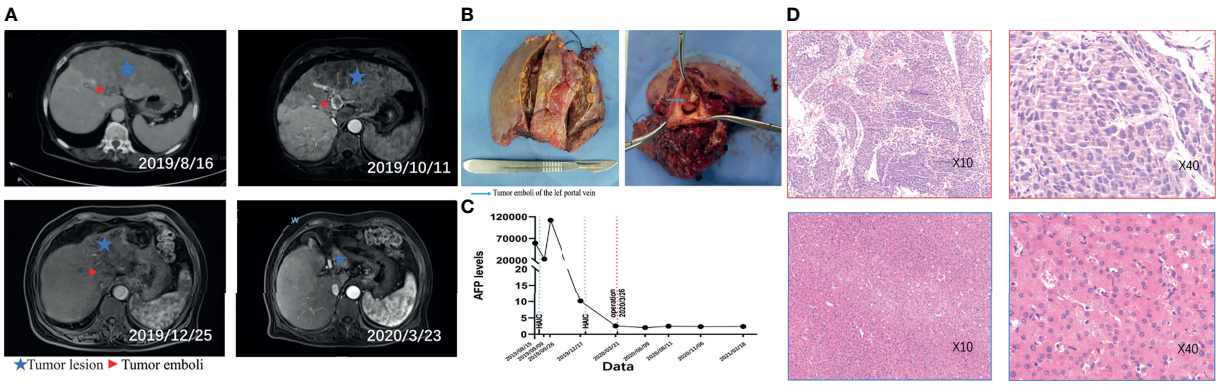

FIGURE 4 | Representative MRI images, AFP levels and pathological findings highlighting changes before and after treatment in one patient. The patient was diagnosed with unresectable HCC in August 2019 and began triple combination therapy. First HAIC was performed on August 27, 2019, and the patient had endoscopic haemostasis for gastrointestinal bleeding (grade 3) that delayed the subsequent HAIC treatment. A second round of HAIC was performed on December 31, 2019, and the patients underwent surgery on March 26, 2020. Preoperative imaging evaluation showed a partial response, and postoperative pathology showed a pathologic complete response. **(A)** Imaging changes before and after treatment (comparison of tumor size at different times was marked by the sagittal segment of the portal vein. Aftertreatment, the volume of the left lobe of the liver was significantly reduced, and the right lobe was compensated); **(B)** intraoperative gross pathological specimen (left), transverse section of left portal vein (right); **(C)** change of AFP levels over time; **(D)** preoperative diagnostic pathological picture (above), postoperative pathological picture (below).

treatment, we applied reduced doses of the therapeutic drugs, appropriately prolonged the treatment period and maintained continuous treatment for patients with poor liver function and poor tolerance of side effects. As the triple combination regimen rapidly reduced the tumor load and retracted the vascular carcinoma thrombus, the liver blood supply improved, increasing tolerance to the treatment. As a result, no grade 4 adverse events occurred and only two patients with portal hypertension experienced gastrointestinal bleeding that resulted in treatment delay.

The reasons for failure to complete treatment according to the protocol were: 1) the occurrence of the COVID-19 pandemic during the treatment period, which made it difficult for patients to seek medical attention, especially trans-provincial patients who could not be treated and re-examined on time; 2) fear of complications of treatment; and 3) financial reasons. Presently, the COVID-19 outbreak has been effectively controlled in China, and the difficulties of patients seeking treatment have been resolved. Regarding complications, the triple combination therapy demonstrated a high level of safety with no grade 4 treatment-related side effects or treatment-related deaths throughout the analysis period. Treatment-related side effects were manageable with symptomatic treatment and adjustment of the drug dosage. For example, gastrointestinal bleeding was safely managed with endoscopic therapy. The inactivation and regression of the main stem thrombus due to the treatment can also reduce the portal pressure and risk of rebleeding. Moreover, for patients with severe gastric fundus varices, endoscopic ligation before treatment can also reduce the risk of severe bleeding. Financially, the triple combination protocol allows for various combinations of different angiogenesis inhibitors and PD-1 inhibitors, which provides patients with options that suit their economic circumstances. Furthermore, our study extended the treatment interval of HAIC to 4–8 weeks, reducing the number of hospitalizations and medical costs, with the efficacy of treatment unaffected.

This study has several limitations. Firstly, this was a single-center, retrospective, single-arm study with a small sample size which is likely to have led to selection bias and does not provide a comparator for the experimental therapy. Prospective studies with a larger population from multiple centers are needed to verify the results. Secondly, patients in this study received different combinations of angiogenesis inhibitors and PD-1 inhibitors. The preliminary conclusion that the different drug combinations were all beneficial warrants further validation in a larger sample. Finally, the baseline characteristics of patients were different for the subgroups with regards to macrovascular invasion, tumor burden, and non-tumor liver histology, and this may have affected the clinical outcome and side effects of treatment.

In conclusion, a triple combination therapy comprised of angiogenesis inhibitors, PD-1inhibitors and HAIC with FOLFOX had a significant therapeutic effect in patients with initially unresectable locally advanced HCC and was associated with an extremely high surgical conversion rate. Toxic effects were manageable, and our findings suggest there will be long-term efficacy.

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 The Research Ethics Committee of Tianjin Medical University Cancer Institute and Hospital. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

TZ: study concept and design. JZ and XZ: acquisition and analysis or interpretation of data. JZ: drafting of the manuscript. TZ and LW: critical revision of the manuscript. JZ and XZ: statistical analysis. WX, HM, and GY: administrative and technical support. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2021.729764/full#supplementary-material

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Lenvatinib Plus Immune Checkpoint Inhibitors Improve Survival in Advanced Hepatocellular Carcinoma: A Retrospective Study

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Background: Nivolumab and pembrolizumab disrupt the programmed cell death-1 immune checkpoint and display promising efficacy and safety results in advanced hepatocellular carcinoma (HCC). However, the benefits remain limited. The preliminary results of lenvatinib (LEN) combined with immune checkpoint inhibitors (ICIs) reveal that the combinations were well-tolerated and encouraging. This study aimed to analyze the safety and efficacy of LEN plus ICIs in a real-world cohort of patients with advanced HCC.

Method: Between June 4, 2017, and June 30, 2019, 16 patients received LEN plus nivolumab, and 13 patients were treated with LEN plus pembrolizumab, with the confirmed advanced HCC retrospectively analyzed. The clinical parameters, as well as the outcomes, were assessed.

Results: All the patients had Barcelona Clinical Liver Cancer Stage C. LEN with ICIs was used as systemic second-, third-, and fourth-line treatments in seven (24.1%), 14 (48.3%), and eight (27.6%) patients, respectively. At the time of data cutoff, six patients (37.5%) were still receiving LEN with nivolumab, while another six patients (46.2%) were still receiving LEN with pembrolizumab. An objective response was recorded in seven patients (25.9%), while the best overall responses were from one complete response and six partial responses. The 6- and 12-month over survival (OS) rates were 62.6% and 53.7%, respectively. Furthermore, the 6- and 12-month progression-free survival (PFS) rates were

43.5% and 31.8%, respectively. In the subgroup analyses, the 6- and 12-month OS and PFS rates for patients treated with LEN plus nivolumab were 62.5% and 52.1%, respectively, and 43.8% and 30.0%, respectively. The 6- and 12-month OS and PFS rates for patients treated with LEN plus pembrolizumab were 51.3% and 51.3%, respectively, and 49.2% and 49.2%, respectively. A total of 11 (31%) deaths were reported in this study, four of which were attributed to grade 5 adverse events presented as fatal treatment-related hepatitis.

Conclusion: The combination of LEN and ICIs is a promising new strategy for the treatment of HCC patients. However, high-grade hepatic toxicity was observed and further evaluation of this combination is still required.

Keywords: hepatocellular carcinoma, lenvatinib, nivolumab, pembrolizumab, survival

INTRODUCTION

Hepatocellular carcinoma (HCC) is the most prevalent primary liver cancer and is ranked as the sixth most common neoplasm, as well as the third leading cause of cancer death (1). However, many patients develop recurrence or disease progression after initial curative surgical or locoregional treatment. At present, there are insufficient therapies that can effectively treat patients with advanced stages of HCC (2, 3). For ten years, the only multikinase inhibitor available for patients with unresectable HCC was sorafenib. Checkmate 459, which is a randomized, multicenter, clinical study, showed that the median overall survival (OS) of the sorafenib group was 14.7 months (4).

In recent years, additional agents, including lenvatinib (LEN) and atezolizumab in combination with bevacizumab, have been introduced to the treatment paradigm as first-line alternatives to sorafenib (5, 6). Similarly, second-line treatment has also evolved, with phase III studies RESORCE, CELESTIAL, and REACH reporting the clinical benefits of regorafenib, cabozantinib, and ramucirumab, respectively, over placebo in patients pretreated with sorafenib (7–9). Immune checkpoint inhibitors have also been examined as novel second-line agents in the treatment of HCC with manageable toxicity in a subset of patients (10, 11). However, phase III studies, in the first-line setting *versus* sorafenib and second-line setting *versus* placebo, have failed to meet their primary endpoints (4, 12).

Although these new inhibitors have improved patient survival, the effectiveness of a single drug remains relatively limited. Furthermore, the benefits remain limited and novel treatment strategies for patients with advanced HCC are urgently required. Numerous studies are examining treatment concepts using combinations of LEN with immune checkpoint inhibitors (ICIs), with preliminary results showing that the combinations were well-tolerated and encouraging (13–15). In 104 patients enrolled in the phase Ib trial of LEN plus pembrolizumab, the confirmed objective response rate (ORR) was 46% and median OS was 22 months (13). Similarly, the phase Ib trial of LEN plus nivolumab revealed manageable adverse events (AEs), and a 76.7% ORR was published in the American Society of Clinical Oncology Seminar in 2020 (16). Based on current data, lenvatinib combined with immunotherapy has shown promising antitumor efficacy and tolerable safety in patients with HCC.

This study aimed to examine the safety and efficacy of LEN plus ICIs in a real-world cohort of patients with advanced HCC treated with sorafenib or more systemic treatment.

PATIENTS AND METHODS

Study Design and Participants

The data of patients with advanced HCC treated with LEN plus ICIs between June 4, 2017, and December 30, 2018, were obtained from the National Cancer Center/ National Clinical Research Center for Cancer/Cancer Hospital and Shenzhen Hospital. All data, including patient history, laboratory results, and radiological information were collected retrospectively.

The diagnosis of HCC was confirmed by histologically or cytologically diagnosis, excluding fibrolamellar, sarcomatoid, and mixed hepatocholangiocellular carcinoma. Patients were required to have measurable disease as defined by the Response Evaluation Criteria in Solid Tumors (version 1.1; RECIST v1.1). The following were the other eligibility criteria: a Child-Pugh score ≤ 7 points, an estimated life expectancy of at least ≥ 12 weeks, an Eastern Cooperative Oncology Group performance status ≤ 2, an absolute neutrophil count ≥ 1.2×10^9/L, a platelet count ≥ 50×10^9/L, serum bilirubin ≤ 2 mg/dL, aspartate aminotransferase (AST) ≤ 5 times the upper limit of normal (ULN), alanine aminotransferase (ALT) ≤ 5 times the ULN, serum prothrombin time ≤ 18 seconds, serum creatinine ≤ 1.5 times the ULN, and measured or calculated creatinine clearance ≥ 60 mL/minute. Untreated hepatitis C virus (HCV) and hepatitis B virus (HBV) patients were eligible, but they had to be on anti-HBV or anti-HCV suppression for ≥ 1 week before receiving ICIs. Programmed Cell Death-Ligand 1 (PD-L 1) expression by immunohistochemistry and tumor mutational burden by genetic sequencing were not assessed regularly. Patients were excluded if they had prior treated with LEN or any ICIs.

The treatment of LEN combined with ICIs was administrated after multidisciplinary discussion, and the chosen of nivolumab or pembrolizumab was open label and non-randomized. Patients received 12 mg (body weight ≥ 60 kg) or 8 mg (body weight ≤ 60 kg) LEN orally once daily. The ICIs were administered as recommended by the official dosage and safety information. Nivolumab was administered intravenously at 3 mg/kg body weight or a fixed dose of 240 mg every two weeks. Pembrolizumab was administered intravenously at a fixed dose of 200 mg every three weeks. Dose delays were determined based on toxicity. Treatment schedules were modified at the discretion of the treating physician, if necessary.

The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki, as reflected in *a priori* approved by the institution's human research committee. The written and informed consent obtained from each patient were included in the study.

End Points and Clinical Assessments

The primary endpoint was the OS rate at 6- and 12-months. Secondary endpoints included AEs, ORR, progression-free survival (PFS). The AEs were assessed using the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI CTCAE; version 4.03). The radiological response was recorded using computed tomography (CT) or magnetic resonance imaging (MRI) at baseline, 6-12 weeks after treatment initiation, and around every 3 months thereafter. The objective response was defined as the proportion of participants with a confirmed complete response (CR) or partial response (PR) assessed with the RECIST v1.1 guidelines using central imaging review (17).

Statistical Analysis

This study was designed as a retrospective cohort study. Patients were followed until their death or last contact, or date of censoring if their death did not occur by the cutoff date of July 16, 2019. Data on baseline characteristics, radiological tumor response, and side effects were summarized using descriptive statistics. The radiological response and time to progression of patients who had at least one follow-up imaging assessment were evaluated. TTP was defined as the time between the date of first checkpoint inhibitor administration and the date of the first radiologically confirmed tumor progression. Data from patients who died without radiologically confirmed tumor progression were censored at the date of their last radiological assessment. PFS was defined as the time from the date of the first checkpoint inhibitor administration until radiological disease progression or death, whichever came first. Patients who were still alive and without radiologically confirmed progression at the date of last contact or data cutoff were censored. OS was defined as the period from the start of immunotherapy until the date of death. Patients who were still alive at the date of last contact or data cutoff were censored. Survival curves were determined using the Kaplan-Meier method and compared using the log-rank test. Statistical analyses were performed using IBM SPSS Statistics version 24.0 (SPSS Inc., Chicago, IL). $P < 0.05$ was considered significant.

RESULTS

Patient Characteristics and Treatment

A total of 29 patients were assessed for eligibility, with all of them taking at least one immunotherapeutic agent combined with LEN. Nivolumab was administered to 16 patients while pembrolizumab was administered to 13 patients. The baseline patient characteristics are summarized in **Table 1**. All the patients had the Barcelona Clinical Liver Cancer (BCLC) Stage C, with 24 (82.8%) patients infected with HBV, two patients infected with HCV, and two patients were infected with both HCV and HBV. LEN with ICIs was used as systemic second-, third-, and fourth-line treatment in 7 (24.1%), 14 (48.3%), and 8 (27.6%) patients respectively, with all patients receiving at least one systemic treatment, such as sorafenib or regorafenib. Furthermore, most patients received local treatment previously, such as hepatectomy, ablation, locoregional radiotherapy, and transhepatic arterial chemotherapy embolization (TACE). The Child-Pugh scores of A, B, C were reported for 79.3%, 13.8%, and 6.9% of patients, respectively. At baseline, 82.8% of patients had macroscopic portal vein invasion and 79.3% had extrahepatic spread.

The median duration of follow-up was 12.0 months (96%CI: 7.5-17.0 months). At the time of data cutoff, six (37.5%) patients were still on treatment with LEN combined with nivolumab, while six (46.2%) were receiving LEN combined with pembrolizumab. The median duration of treatment for ICIs was 10.5 months (95% CI: 7.53-12.97 months), nivolumab was seven months (95% CI: 3.19-11.38 months), and pembrolizumab was one month (95% CI: 0.67-2.5 months). The most common reasons for treatment discontinuation were progressive disease (PD) in 11 (37.9%) patients and serious AEs in five (17.2%). After PD, seven participants went on to receive an alternative treatment: one received a single LEN, five received regorafenib, and one received the PD-L1 immune checkpoint.

Due to fatal treatment-related adverse events, two participants in LEN plus nivolumab did not have any assessment data after baseline. An objective response was recorded in seven (25.9%) of the 29 participants who received at least one dose of ICIs. Among the seven responders, the best overall responses were one CR and six PR. Furthermore, 12 (44.5%) participants had stable disease (SD), while eight (29.6%) had PD. The disease control rate (DCR) was reported in 19 (70.4%) of the 27 treated participants (**Table 2**). At the time of data cutoff, six of the seven responses were ongoing, and the median duration of response (DOR) was seven months (95% CI: 1.19-12.81 months). In this study, 11 (40.7%) of the 27 participants died, the median TTP was 7 months (95% CI: 3.44-10.56 months) (**Table 2** and **Figure 1**), the 6- and 12-month OS rates were 62.6%, and 53.7% (**Table 2** and **Figure 2**), respectively, and the 6- and 12-month PFS rates were 43.5% and 31.8% (**Table 2** and **Figure 3**), respectively.

At least one adverse event was reported among the 24 (82.8%) participants: grade 1-2 in 12 (41.4%) patients, grade 3 in six (20.7%) patients, grade 4 in two (6.9%) patients, and grade 5 in four (13.8%) patients (**Table 3**). The following were the most common treatment-related AEs of any grade in the participants: increased ALT concentration in 14 (48.3%), increased AST

TABLE 1 | Baseline characteristics.

	LEN + NIVO n = 16	LEN + PEM n = 13	All patients n = 29
Age(y), mean ± SD	49 ± 3.87	57.5 ± 4.22	42.5 ± 3.57
Sex			
Male	4	6	10
Female	12	7	19
Aetiology			
Hepatitis B	14	10	24
Hepatitis C	1	1	2
Hepatitis B+C	1	1	2
Other	0	1	1
Prior treatment			
Hepatectomy	9	6	15
Ablation	3	2	5
Loco-regional (TACE/radiation)	10	11	21
Previous sorafenib	16	13	29
Previous regorafenib	12	10	22
Previous Anlotinib	3	5	8
LEN+ICIs			
Second-line	4	3	7
Third-line	9	5	14
Fourth-line	3	5	8
Macrovascular invasion	13	11	24
Extrahepatic metastasis	13	10	23
Child-Pugh stage			
A	12	11	23
B	2	2	4
C	2	0	2
ECOG PS			
0	13	10	23
1-2	3	3	6
Alpha-Fetoprotein			
<400 (IU/ml)	6	7	13
≥400 (IU/ml)	9	6	15

TACE, Transhepatic arterial chemotherapy embolization; LEN, Lenvatinib; ICIs, Immune checkpoint inhibitors; BCLC, Barcelona Clinical Liver Cancer Stage.

concentration in 13 (44.8%), and hyperlipemia in 13 (44.8%), nausea in 7 (24.1%), proteinuria in 7 (24.1%), decreased appetite in 7 (24.1%), rash (7 [24.1%]), diarrhea in 6 (20.7%), and asthenia in 6 (20.7%). Treatment-related events of grade 3 or higher severity were reported in 12 (41.4%) participants. The most common grade 3 events were increased ALT concentration, which was observed in 4 patients (13.8%), and elevated AST concentration in three patients (10.3%). Three grade 4 occurrences had allergic reactions, as well as increased ALT and AST concentrations. Among the 29 participants, 4 (13.8%) of them had dose interruptions due to three participants treated with LEN and nivolumab having hepatitis, while the other one treated with LEN and pembrolizumab had severe edema. The three participants in the nivolumab group continued treatment after their hepatitis was cured. Despite the use of systemic corticosteroids for the management of AEs, patients continued to experience clinical benefits. Out of the three patients re-challenged after receiving systemic corticosteroid for AEs, two participants had partial responses, while the third had disease progressive.

In this study, 11 (31%) deaths were reported in the study, four of which were attributed to grade 5 adverse events that resulted in fatal immune-related hepatitis. The median time of fatal toxic effects typically occurred in 0.5 ± 1.89 months and the median time from symptom onset to death was five days (range, 1-9 days). Liver protection and prednisone therapy failed to reverse the liver injury, and the dysfunction progressed to liver failure.

In the subgroup analyses, ORR was represented in six (42.8%) of nivolumab and one (7.7%) of pembrolizumab. LEN plus nivolumab had the best ORR with one CR (7.14%), five PR (35.7%), and four SD (28.6%). LEN plus pembrolizumab had only one PR (7.7%) and 8 SD (61.5%). DCR was reported in ten (71.4%) of the LEN plus nivolumab group and nine (69.2%) of the LEN plus pembrolizumab group, respectively (**Table 2**). The 6- and 12-month PFS rates for patients treated with LEN plus nivolumab were 43.8% and 30.0%, while for patients treated with LEN plus pembrolizumab they were 49.2% and 49.2%, respectively (**Figure 4**). The 6- and 12-month OS estimates for the LEN plus nivolumab group were 62.5% and 52.1%, respectively, and 51.3% and 51.3%, respectively, for the LEN plus pembrolizumab group (**Figure 5**). In terms of safety, the number of patients who developed any grade (Group nivolumab

TABLE 2 | Radiological response according to RECIST1.1 and survival.

	LEN + NIVO n = 16	LEN + PEM n = 13	All patients n = 29
Best response			
CR	1 (6.3%)	0	1 (3.4%)
PR	5 (31.2%)	1 (7.7%)	6 (20.7%)
SD	4 (25%)	8 (61.5%)	12 (41.4%)
PD	4 (25%)	4 (30.8%)	8 (27.6%)
Not evaluable	2 (12.5%)	0	2 (6.9)
ORR (CR+PR)	6 (37.5%)	1 (7.7%)	7 (24.1%)
DCR (CR+PR+SD)	10 (62.5%)	9 (69.2%)	19 (65.5%)
TTP, median (95% CI)	7 (95% CI 0.39-13.61)	–	7 (95% CI 3.44-10.56)
DOR (range, months)	7 (3-11)	–	7 (3-11)
6-months PFS rate	43.8%	49.2%	43.5%
12-months PFS rate	30.0%	49.2%	31.8%
6-months OS rate	62.5%	51.3%	62.6%
12-months OS rate	52.1%	51.3%	53.7%

CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease; ORR, objective response rate; DCR, disease control rate; TTP, time to progression; DOR, duration of response; PFS, progression-free survival; OS, over survival.

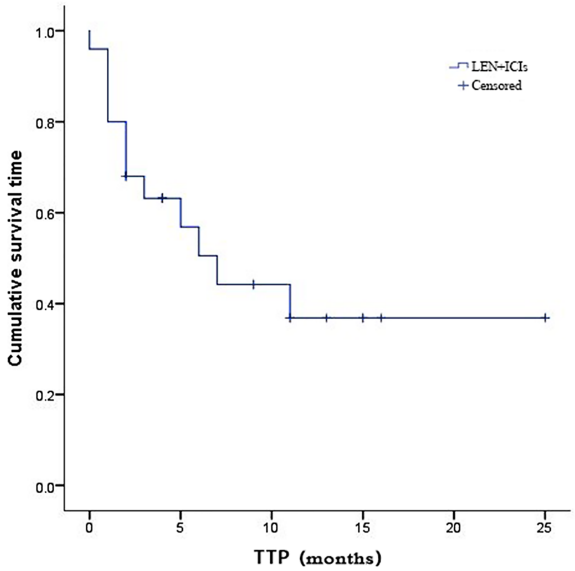

FIGURE 1 | Kaplan-Meier estimates of time to progression for 27 eligible patients with advanced hepatocellular carcinoma who were treated with lenvatinib plus immune checkpoint inhibitors.

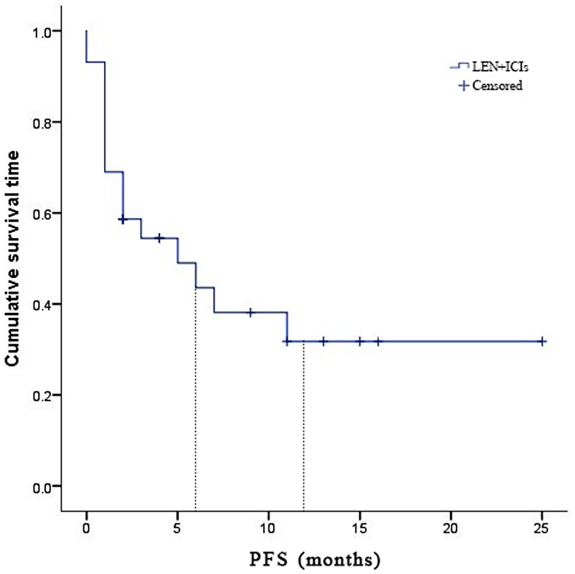

FIGURE 3 | Kaplan-Meier estimates of progression-free survival for 27 eligible patients with advanced hepatocellular carcinoma who were treated with lenvatinib plus immune checkpoint inhibitors.

vs pembrolizumab, n = 15 [93.8%] *vs* n = 9 [69.2%]) or high-grade (Group nivolumab *vs* pembrolizumab, n = 7 [43.8%] *vs* n = 5 [38.5%]) adverse events was similar between LEN plus nivolumab or pembrolizumab, with both groups having the same adverse reaction spectrum (**Table 3**).

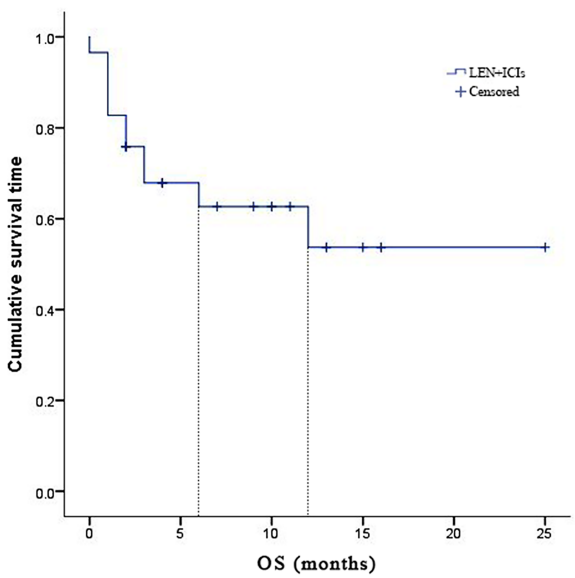

FIGURE 2 | Kaplan-Meier estimates of over survival for 27 eligible patients with advanced hepatocellular carcinoma who were treated with lenvatinib plus immune checkpoint inhibitors.

DISCUSSION

In second-line trials involving patients who have failed sorafenib, the OS in the placebo group is around 8 months (9, 18, 19). The sequential molecular targeting agent treatment further improved prognosis (20), but late line patients had a worse status and more complex tumor resistance, leading to poor survival during progression after previous systemic therapy. Therapeutic decisions for late-line patients are mainly determined based on the tumor stage and the underlying liver dysfunction.

Several single options evaluated the efficacy and safety of late line HCC compared to the best supportive care or placebo (12, 21, 22). For example, LEN prolonged OS, offering safety and tolerability in first-line treatment (5), as well as providing a good sequential treatment option after progression in the third line of unresectable HCC patients with better hepatic reserve function (21). Furthermore, nivolumab was found to be safe in patients with Child-Pugh class B liver dysfunction (22). In addition, pembrolizumab has demonstrated encouraging antitumor activity and was well tolerated in the Asian subgroup when used as a second-line treatment for advanced HCC (23).

Although a significant number of patients had objective responses and median PFS and OS that were both promising after treatment with LEN or ICIs, the benefits remain limited. Numerous ongoing studies are examining regimens combining LEN with ICIs, with preliminary results revealing that the treatment was well-tolerated and encouraging. There is currently no late-line data on advanced HCC using the combination of LEN and ICIs.

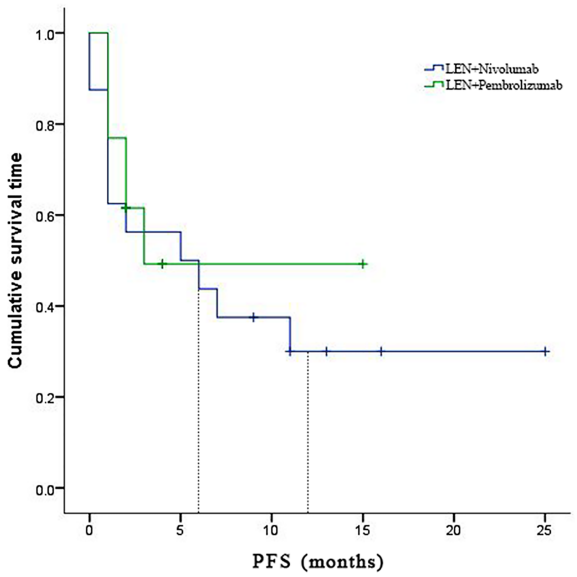

FIGURE 4 | Kaplan-Meier estimates of over survival with advanced hepatocellular carcinoma who were treated with lenvatinib plus Nivolumab and pembrolizumab, respectively.

In this study, the combination of LEN with programmed cell death protein 1 (PD-1) targeted immunotherapy demonstrated promising clinical efficacy in a real-world cohort of patients with advanced HCC. A substantial number of objective responses (24.1%) and a DCR of 65.5% were discovered in the 29 treated participants, who were consistently observed across several risk factors associated with the prognosis of advanced HCC. The responses were generally positive; the 6- and 12-month OS rates were 62.6% and 53.7%, respectively; median TTP was seven months (95% CI 3.44-10.56 months); the 6- and 12-month PFS rates were 43.5% and 31.8%, respectively. These findings indicated that the combination of LEN with nivolumab or pembrolizumab could provide an effective treatment option in a late-line systemic therapy setting. In preclinical murine models, the combination of LEN with the anti-PD-1 antibody has been shown to enhance antitumor activity. LEN significantly decreased the population of tumor-associated macrophages, as well as increased the percentage of activated CD8+ T cells secreting interferon-γ+ and granzyme B (24, 25). In addition, LEN significantly reduced the level of tumor programmed death-ligand 1 (PD-L1) and Treg differentiation, improved anti-PD-1 efficacy by blocking FGFR4, and inhibiting TGFß signaling (26, 27). The extent to which combination therapies pose clinical safety and tolerability challenges, and whether these challenges will limit their usefulness as an anticancer therapy, have been the focus of an increasing number of studies.

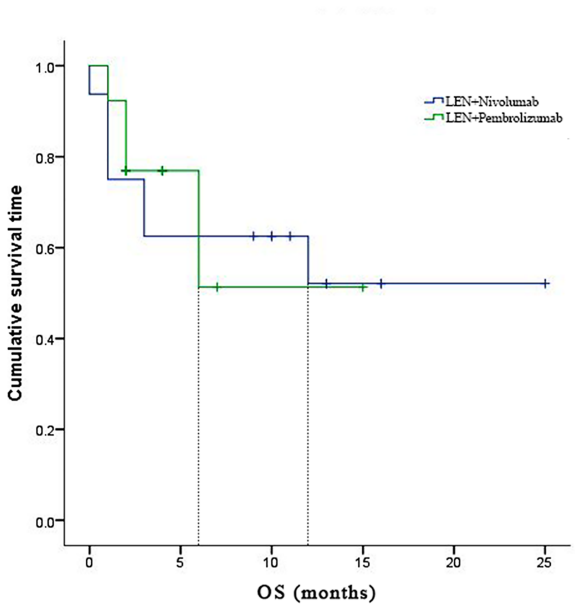

FIGURE 5 | Kaplan-Meier estimates of progression-free survival with advanced hepatocellular carcinoma who were treated with lenvatinib plus Nivolumab and pembrolizumab, respectively.

Recently, the preliminary results of LEN combined with nivolumab or pembrolizumab were reported in first-line treatment evaluating the safety and effectiveness in advanced HCC. The combination of LEN and nivolumab showed a promising ORR of 76.7% and DCR of 96.7% by modified RECIST (16), and the safety was assessed in another trial (28). Meanwhile, the combination of LEN and pembrolizumab showed an encouraging ORR of 36%, and DCR of 88% by RECIST v1.1 (13). In the subgroup of our study, LEN combined with nivolumab had an objective response of 37.5%; DCR was 62.5%; the 6- and 12-month PFS rates were 43.8% and 30.0%, respectively; the 6- and 12-month OS rates were 62.5% and 52.1%, respectively. A combination of LEN and pembrolizumab had a 7.7% ORR and a 62.5% DCR, while the 6- and 12-month overall survival estimates were 51.3% and 51.3%, respectively. Despite the poor prognosis of this population, six patients (20.7%) experienced durable and ongoing confirmed radiographic responses, including one patient who had a complete response at the time of the last follow-up. Future studies assessing the PD-1 score and next-generation tumor sequencing may help in identifying markers of potential responders. In this study, the combination of lenvatinib plus ICIs improved both disease control and survival.

There were no new or unexpected toxicities resulting from the combination of lenvatinib with nivolumab or pembrolizumab (13, 16, 28). The number of discontinuations due to treatment-related AEs was 13.8%, and treatment-related events, such as increased ALT or AST concentration, hyperlipemia, nausea, proteinuria, decreased appetite, rash, diarrhea, and asthenia (events that typically occur following treatment), were observed in more than 10% of participants. Although >80% of subjects experienced AEs, the majority of them were associated with complications of comorbid liver dysfunction and advanced tumor burden, as previously reported in studies on patients with

TABLE 3 | Adverse events.

	LEN + NIVO n = 16		LEN + PEM n = 13		All patients n = 29	
	Any grade	Grade ≥ 3	Any grade	Grade ≥ 3	Any grade	Grade ≥ 3
Rash	3 (18.7%)	–	3 (23.07%)	–	6 (20.7%)	–
Pruritus	2 (12.5%)	–	1 (7.69%)	–	3 (10.34%)	–
Fatigue	3 (18.7%)	–	1 (7.69%)	–	4 (13.79%)	–
Vomiting	3 (18.7%)	–	2 (15.38%)	1 (7.69%)	5 (17.24%)	2 (6.9%)
Diarrhoea	4 (25%)	–	2 (15.38%)	–	6 (20.7%)	–
Paresthesia	–	–	1 (7.69%)	–	1 (3.45%)	–
Arthritis	1 (6.25%)	–	1 (7.69%)	–	2 (6.9%)	–
Thyroiditis	2 (12.5%)	–	1 (7.69%)	–	3 (10.34%)	–
Dyspnea	1 (6.25%)	–	–	–	1 (3.45%)	–
Abdominal pain	2 (12.5%)	1 (6.25%)	–	1 (7.69%)	4 (13.79%)	2 (6.9%)
Nausea	7 (43.75%)	–	3 (23.07%)	–	10 (34.48%)	–
Allergic reaction	–	–	1 (7.69%)	–	1 (3.45%)	–
Gastric ulcer	1 (6.25%)	–	–	–	1 (3.45%)	–
Decreased appetite	5 (31.25%)	1 (6.25%)	2 (15.38%)	–	7 (24.14%)	–
Hyperlipasaemia	8 (50%)	–	5 (38.46%)	–	13 (44.8%)	–
Asthenia	4 (25%)	1 (6.25%)	2 (15.38%)	–	6 (20.7%)	–
Myelosuppression	2 (12.5%)	–	–	–	2 (6.9%)	–
Amylase/Lipase increase	1 (6.25%)	–	–	–	1 (3.45%)	–
AST increase	10 (62.5%)	6 (37.5%)	3 (23.07%)	2 (15.38%)	13 (44.8%)	8 (27.6%)
ALT increase	11 (68.75%)	7 (43.75%)	3 (23.07%)	2 (15.38%)	14 (48.28%)	9 (30.03%)
Proteinuria	4 (25%)	–	3 (23.07%)	1 (7.69%)	7 (24.14%)	1 (3.45%)

AST, aspartate aminotransferase; ALT, alanine aminotransferase.

Child-Pugh class B HCC (29). Treatment-related grade ≥3 events were reported to have occurred in 41.4% of patients.

Among the three of the five patients who received systemic corticosteroid for AEs when re-challenged; two had partial responses, while the other had disease progression. The incidence of AEs with immunotherapeutic agents indicated an active immune status, suggesting that there were potential clinical benefits to the patient (30).

In the largest retrospective evaluation of fatal ICIs-associated toxic effects published by the World Health Organization (WHO) pharmacovigilance database (Vigilyze), hepatitis accounted for around 20% of deaths of reported anti-PD-1/PD-L1 related fatalities (31). In the Checkmate-040 study, 22–30% of the patients receiving nivolumab had an increase in ALT/AST levels. A similar rate was also described in the Keynote-224 study of pembrolizumab. This further validated the recently published data of nivolumab in Child-Pugh B patients, where treatment of related hepatic AEs was described in only four out of 49 patients, resulting in treatment discontinuation of two patients in this cohort (32). The most common grade 3/4 immune-mediated AEs in this cohort was liver toxicity, with four deaths attributed to grade 5 AEs presenting fatal treatment-related hepatitis. It was discovered that these events generally occurred very early on after therapy initiation and the duration from symptom onset to death was short; nevertheless, it was unclear how the rates of fatal toxic effects contributed to the combination with lenvatinib. Due to the extremely high prevalence of ICI usage, more aggressive combinations that are in development will cause an increase in life-threatening and fatal complications. Therefore, the potential increased risk of liver toxicity must be taken into account in clinical management.

Despite the retrospective nature and the lack of a control group, the strength of this study is the provision of unique real-world data on multiple lines of a systemic pretreatment patient cohort that is excluded from clinical trials. These findings contribute new, important information on LEN plus ICIs in advanced HCC, particularly the first subgroup report on LEN plus nivolumab.

There are several limitations to this study. Firstly, this study is of a retrospective nature which could influence patient selection bias. Therefore, the results must be interpreted with caution due to the heterogeneous nature of the study population and different treatment regimens. Secondly, the size of the cohort samples was relatively small, reducing the quality of the conclusions reached. Thirdly, due to a lack of detection of PD-L1 expression on tumor cells, future studies will require the evaluation of the PD-1 and PD-L1 expression levels on tumor-infiltrating lymphocytes as potentially valuable biomarkers. In addition, a longer follow-up is required for more meaningful median overall survival results in the cohorts. Finally, the study was not designed to statistically compare the clinical outcomes of lenvatinib plus nivolumab against lenvatinib plus pembrolizumab, and further studies in larger populations are warranted.

CONCLUSION

The combination of immunotherapy and targeted therapies has attracted a huge amount of interest in the field, increasing hopes that novel, effective therapeutic options will become soon available, leading to new strategies for the management of HCC patients. However, high-grade hepatic toxicity was observed, which required further evaluation of this combination.

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

XH, ZH, LX, and XC contributed to conception and design of the study. TM, YR, YN, and XY contributed to the acquisition, analysis, or interpretation of data for the work. XH and XB wrote the first draft of the manuscript. All authors contributed to the article and approved the submitted version.

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REVIEW
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Downstaging Conversion Therapy in Patients With Initially Unresectable Advanced Hepatocellular Carcinoma: An Overview

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The high mortality rate associated with hepatocellular carcinoma (HCC) is partly due to the high proportion of patients who present with advanced stage disease at diagnosis, for whom there are limited treatment options. For selected patients with initially unresectable HCC, locoregional and/or systemic treatments can result in tumor downstaging and consequently provide opportunities for surgical intervention and the potential for long-term survival. Therefore, the key aim of 'conversion therapy' is to reduce tumor burden so that patients become amenable to surgical resection. Various therapies have been investigated as candidates for downstaging patients with potentially resectable HCC including transarterial chemoembolization, transarterial radioembolization with yttrium-90 microspheres, radiotherapy, systemic therapies and combination or multimodality treatment approaches. However, downstaging conversion therapy remains controversial and there are several challenges such as defining the criteria used to identify the population of patients who are 'potentially resectable', the criteria used to define successful downstaging, and the optimum treatment approach to maximize the success of downstaging therapy. In this review article, we summarize clinical experience and evidence of downstaging conversion treatment in patients identified as having 'potentially resectable' HCC.

Keywords: hepatocellular carcinoma, downstaging, conversion therapy, initial unresectable, systemic

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INTRODUCTION

Worldwide, liver cancer is the sixth most commonly diagnosed cancer, with an estimated 905,677 new cases and 830,180 deaths in 2020 (1). The incidence rates associated with liver cancer are 2-3 times higher in men versus women (14.1 versus 5.2 per 100,000 individuals), with an overall mortality rate of 8.7 per 100,000 individuals (1). In China, Liver Cancer is the fourth most commonly diagnosed cancer, behind lung, stomach, and breast cancers, with 410,038 new cases in 2020 and 391,152 deaths (2). Similar to the global epidemiology, age-standardized incidence rates of liver cancer in China are higher in men than women (27.6 versus 9.0 per 100,000 individuals) and associated with an overall mortality rate of 17.2 per 100,000 individuals. Thus, both incidence and

mortality rates for liver cancer are approximately 2-fold higher in China than global estimates (2). In addition, the estimated 5-year survival rate for Chinese patients with HCC is 12.2% (3).

The high mortality rate associated with HCC is partly due to the high proportion of patients who present with advanced disease, for whom there are limited treatment options (4). Surgical treatment provides the best opportunity for achieving long-term survival in HCC patients and is mainly comprised of hepatectomy and liver transplantation. For unresectable HCC, the application of preoperative treatments such as transarterial chemoembolization (TACE) may result in tumor downstaging and consequently provide initially ineligible patients with opportunities for surgical intervention. Improved long-term survival may be achieved in HCC patients undergoing resection after downstaging.

Downstaging conversion therapy is an emerging treatment approach for HCC that aims to reduce tumor burden using locoregional or systemic therapy so that patients become amenable to surgical resection. This type of preoperative therapy in HCC is controversial. However, evidence is accumulating to suggest that successful downstaging therapy followed by surgical resection is achievable in a subpopulation of patients. The criteria used to identify the population of patients who are 'potentially resectable', the criteria used to define successful downstaging, and the optimum treatment approach to maximize the success of downstaging therapy are all factors that remain subject to ongoing debate. This article will overview clinical experience to date with downstaging conversion treatment in patients identified as having 'potentially resectable' HCC. We also review clinical trials conducted in patients with unresectable HCC in which notable tumor responses were achieved, even if eligibility for resection was not reported as a treatment outcome.

CONVERSION THERAPY: TARGET POPULATION AND PRINCIPLES

Conversion Therapy – Target Patient Population

The causes of unresectable liver cancer can be divided into surgical causes and oncological causes. Surgical causes refer to the inability to perform safe surgical excision due to a patient's inability to withstand surgery because of their general condition, liver function or insufficient remaining liver volume. Oncological causes refer to predicted efficacy after excision failing to surpass other, non-surgical treatment methods (5). There is a consensus for the definition of surgically unresectable liver cancer, while oncologically unresectable liver cancer is less well defined. The goal of conversion therapy is to eliminate these two causes, so as to achieve the conversion from unresectable liver cancer to resectable liver cancer (5).

While the Barcelona Cancer Liver Clinic (BCLC) staging system for liver cancer is employed extensively throughout the US and Europe, in China the China Liver Cancer Staging (CNLC) system is preferred because of its relevance to local systems and practices (6). In general terms, the CNLC system divides each stage from the BCLC into two substages, with BCLC stages 0/A, B and C translating to CNLC stages Ia, Ib, IIa, IIb, IIIa and IIIb. BCLC stage 0/A is considered very early or early HCC and the equivalent CNLC stages Ia and Ib are defined as single nodules ≤5 cm or >5 cm, respectively; stage Ib also includes the presence of 2-3 nodules ≤3 cm. The intermediate BCLC B stage is represented within the CNLC system as stages IIa (2-3 nodules >3 cm) and IIb (≥4 nodules). The BCLC stage C, defined as advanced disease, is represented in the CNLC system by stages IIIa (vascular invasion) and IIIb (extrahepatic metastases) (6–8) (**Figure 1**).

Surgically unresectable CNLC-stage Ia, Ib, IIa liver cancer (considered unresectable mainly due to the patient's general condition or liver function intolerance, insufficient remaining liver volume or insufficient resection margins) and surgically resectable CNLC-stage IIb and IIIa liver cancer (with limited tumor burden) are potentially resectable liver cancers, and multi-mode, high-intensity treatment strategies can be explored and adopted to facilitate the conversion. For surgically unresectable CNLC- stage IIb and IIIa liver cancer (for which the predicted surgical efficacy does not surpass other non-surgical treatment options), it is recommended that the current treatment norms should be followed and a gradual treatment strategy should be adopted, both the intensity and safety of treatment should be taken into account, and surgical excision should be conducted when applicable.

Conversion therapy may be considered distinct from neoadjuvant therapy. While both treatment approaches are administered as peri-surgical procedures and utilize the same modalities, they have different objectives. Neoadjuvant therapy is administered to patients with resectable disease to decrease tumor size prior to definitive surgery. In contrast, conversion therapy is administered to patients with initially unresectable disease who are considered potentially resectable after successful downstaging. However, when the treatment is applied to the patients with surgically resectable but oncologically unresectable HCC, both treatments may be overlapped in the target population (eg. surgically resectable CNLC-IIb or IIIa) or treatment objectives, eg. to change some oncological factors, such as tumor thrombi, satellite nodules, or even microvascular invasion. Basically, neo-adjuvant therapy is to decrease tumor burden or other oncological factors to improve the outcome after surgical resection, so neo-adjuvant therapy can be considered as oncological conversion therapy.

Principles of Conversion Therapy

Conversion treatment strategies should be developed under the guidance of a multidisciplinary team, including surgeons, medical oncologists, interventional radiologists and diagnostic radiologists, or other related doctors. Conversion therapy planning should take multiple factors into account including liver function, liver function reserve, the number, location and size of liver lesions, vascular invasion, comorbidities, and the specific objectives of treatment. An ideal conversion treatment should have a high objective response rate and less adverse effects on patients and following surgical operation, and strive to achieve conversion in as short a timeframe as possible. During conversion therapy, the response to treatment should be closely

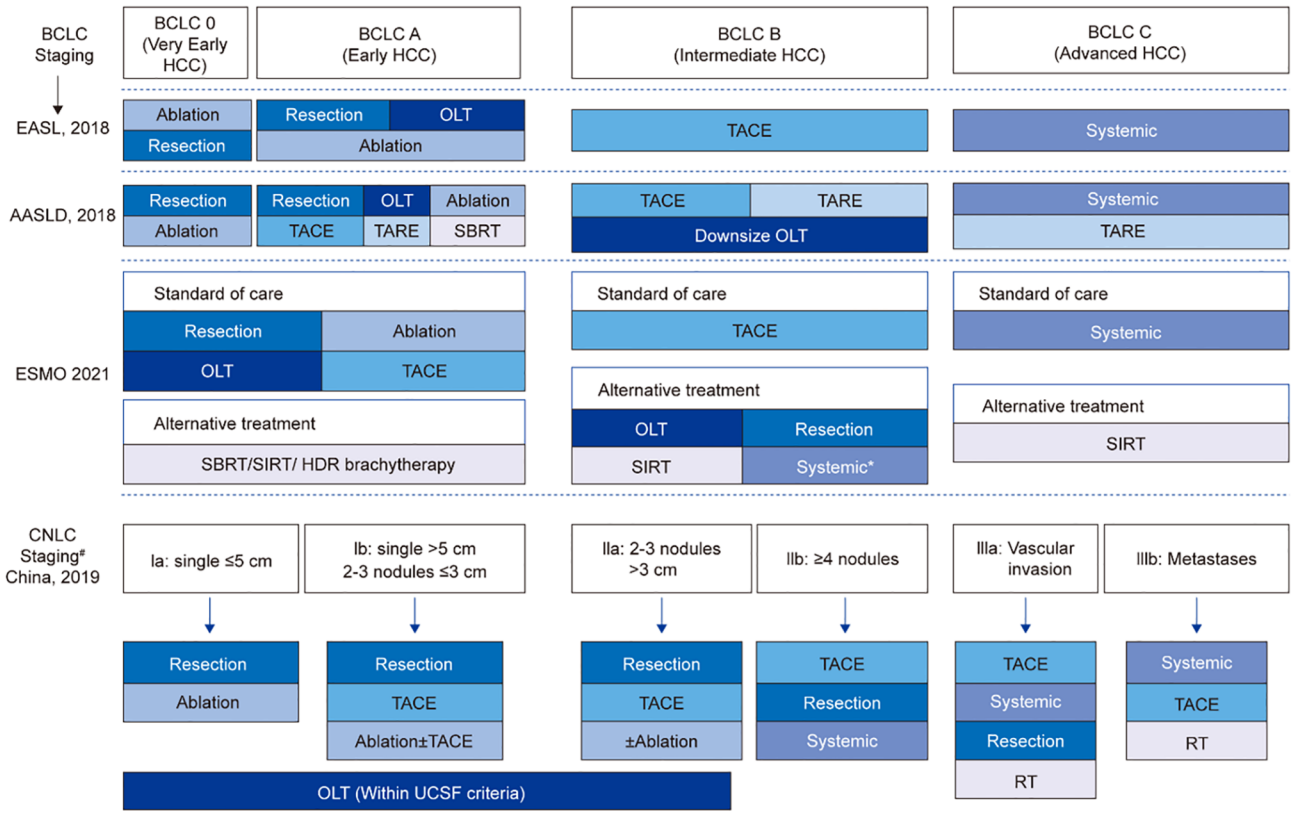

FIGURE 1 | Comparisons of staging and treatment algorithms of HCC among 2021 ESMO, 2018 EASL, 2018 AASLD, and 2019 Chinese guidelines. *For patients who are not suitable for local therapies; #If PS = 0-2, CNLC stage I-III; if PS > 2, CNLC stage IV; BCLC, Barcelona Clinic Liver Cancer; EASL, European Association for the Study of the Liver; AASLD, American Association for the Study of Liver Diseases; CNLC, China liver cancer staging; OLT, orthotopic liver transplantation; TACE, transarterial chemoembolization; TARE, transarterial radioembolization; SBRT, stereotactic body radiotherapy; SIRT, selective internal radiotherapy; RT, radiation therapy; UCSF, University of California San Francisco.

monitored, and the timing of surgery should be determined based on a judgment of predicted efficacy, although an objective evaluation is needed to facilitate a good judgment.

Conversion therapy for downstaging or downsizing HCC can utilize many of the treatment modalities. Various therapies that have been studied as candidates for conversion therapy include TACE, transarterial radioembolization (TARE) with yttrium-90 microspheres (Y90), systemic therapies, and combination or multimodality treatment approaches (**Table 1**). An early literature review suggests that 8-18% of patients presenting with unresectable HCC may be suitable for salvage surgical resection after initial palliative treatment to downstage the tumor (23).

LOCOREGIONAL TREATMENT FOR CONVERSION THERAPY

Transarterial Chemoembolization

TACE is the practice of delivering a chemotherapy agent directly to a liver tumor through the hepatic blood supply, usually the hepatic artery (24). To reduce leakage of the chemotherapy into the systemic circulation, microspheres loaded with chemotherapy have been developed (drug-eluting bead-TACE [DEB-TACE]). DEB-TACE permits sustained elution of the chemotherapy at the site of the tumor coupled with reduced systemic concentrations, allowing use of relatively high dose levels and/or frequency (24).

Data from a retrospective analysis show that patients who achieve tumor downstaging following TACE and then undergo surgical resection achieve better outcomes than patients who achieve downstaging but do not undergo surgery (9). Of the 831 patients with unresectable HCC included in this analysis who received TACE as initial treatment, 82 achieved significant downstaging and became eligible for resection. Of the patients eligible for surgery, 43 received salvage resection and 39 declined surgery. The majority of patients had an ECOG performance score 0-1 (91% and 87%) and were Child-Pugh class A (95% and 92%) in the group that received salvage resection and declined surgery respectively. Median overall survival (OS) was higher for patients who received surgery compared with those who declined (49 months versus 31 months P=0.027). A significant survival benefit favoring surgery was also achieved in subgroups of patients with macroscopic vascular invasion and with a partial response to TACE (9).

TABLE 1 | Summary of clinical evidence for tumor down-staging in patients with unresectable HCC.

Ref	Study design	Treatment(patient No.)	ORR (%)	Downstaging rate or subsequent surgical rate (%)	Outcome
Zhang et al., 2016 (9)	Retrospective; single center; consecutive patients	TACE (831)	–	Downstaging rate: 9.9%	2-year OS rate: 93%
Fan et al., 1998 (10)	Retrospective	TACE (65)	–	100	5-year OS rate: 56%
Labgaa et al., 2019 (11)	Retrospective	TARE (349)	–	Subsequent OLT/LR rate: 9%	5-year OS rate:86%
Tabone et al., 2020 (12)	Retrospective; single center; consecutive patients	TARE (24)	–	20.8%	–
Inarrairaegui et al., 2012 (13)	Retrospective	TARE (21)	–	28.6%	–
Lewandowski et al., 2009 (14)	Single-center; comparative study	TACE vs TARE (78)	71% vs 86%	31% in the TACE group and 58% in the TARE group	–
Zeng et al., 2002 (15)	Retrospective	Radiation; hepatic artery ligation plus RIT vs TACE plus EBRT (67)	72% vs 86%	53% vs 23%	–
Lee et al., 2014 (16)	Retrospective consecutive patients	Concurrent chemo/radiotherapy (264)	–	6.8%	Curative resection group: 49.6% at 5-year survival
He et al., 2019 (17)	Randomized, open-label	Sorafenib vs sorafenib plus HAIC (247)	–	12.8%vs 0.8%	13.7m vs 7.13m
Chong et al., 2018 (18)	Retrospective	Concurrent chemoradiotherapy (CCRT) followed by HAIC	–	26.5%	–
Zhu et al., 2021 (19)	Single center retrospective study	TKI+PD-1	–	15.9%	–
Zhang et al., 2021 (20)	Single center retrospective study	TKI+PD-1+TACE	96%	56%	–
He et al., 2021 (21)	Randomized, open-label	Lenvatinib+PD-1+HAIC (LeToHAIC) vs. Lenvatinib	LeToHAIC Group: 67.6% (mRECIST)	LeToHAIC group: 12.7%	NR
Zhang et al., 2020 (22)	Prospective real-world study	Lenvatinib + PD-1	45.5% (mRECIST)	Conversion rate: 42.4%, surgical rate: 30.3%	NR

cTACE, conventional transarterial chemoembolization; DEB, drug-eluting bead; EBRT, external beam radiotherapy; HAIC, hepatic arterial infusion of chemotherapy; HCC, hepatocellular carcinoma; OLT, orthotopic liver transplant; ORR, objective response rate; OS, overall survival; PFS, progression-free survival; RT, radiotherapy; RIT, radioimmunotherapy; TAE, transarterial embolization; TACE, transarterial chemoembolization; TARE, transarterial radioembolization. NR, not reached.

Several other studies have also indicated that TACE may be effective for downstaging tumors. In a retrospective study of patients with initially unresectable HCC who underwent hepatic resection following TACE, complete pathological tumor necrosis was achieved in 11 of 65 patients (16.9%) (10). Sixty-one patients in this series underwent resection, and 1-, 3- and 5-year survival rates were 80.0%, 65.0% and 56.0%, respectively (10). In addition, Li and colleagues reported that preoperative TACE did not impact perioperative morbidity or mortality in a multicenter, propensity matching analysis of patients with advanced HCC, 90.7% of whom had Child-Pugh class A liver function (25). Conversely, this study reported that preoperative TACE was associated with improved OS and relapse-free survival after liver resection in patients with large HCC (≥10 cm) (25).

The prospective randomized PRECISION V study compared response rates in patients receiving transarterial doxorubicin delivered *via* conventional TACE versus DEB-TACE (26). The ECOG performance score ratio (0/1) and Child-Pugh classification ratio (A/B) were similar (80/28 Vs. 74/19 and 89/19 Vs. 77/16) between the groups receiving TACE versus DEB-TACE, respectively. At 6 months, DEB-TACE was associated with significantly higher rates of complete response compared with conventional TACE (27% versus 22%), contributing to a higher overall response rate (ORR) for patients receiving DEB-TACE (52% versus 43%). In addition, the improved response rates among patients receiving DEB-TACE were more pronounced in the subgroup of patients with advanced disease. This study did not assess post-treatment eligibility for resection and therefore the translation of response rates into resectability is unclear. While subsequent studies have assessed DEB-TACE as a bridging therapy prior to liver transplant (27–29), the role of this treatment modality as a conversion therapy prior to surgical resection requires further confirmation.

Hepatic Artery Infusion Chemotherapy

Interestingly, interim results from a phase 3 study of neoadjuvant HAIC with FOLFOX in patients with resectable HCC (BCLC stage A or B) showed that HAIC monotherapy can reduce the incidence of microvascular tumor thrombi, and this may suggest a role for HAIC monotherapy as part of a conversion therapy strategy (30). A study of patients with HCC and portal vein invasion who received either sorafenib or sorafenib in combination with hepatic arterial infusion of chemotherapy (HAIC; oxaliplatin, fluorouracil and leucovorin) indicated that the combination modality may have potential as a downstaging approach for patients with potentially resectable disease (17). In this study, 16 of 125 patients receiving sorafenib plus HAIC subsequently underwent curative resection and three patients achieved a pathologic complete response. Furthermore, resection was also possible in one patient with initial disease progression on sorafenib alone, who then crossed

over to the combination arm (17). Another retrospective study comparing lenvatinib monotherapy with lenvatinib combined with toripalimab and HAIC (LeToHAIC) for the treatment of advanced HCC, in patients with an ECOG score of 0 or 1, found that LeToHAIC combination therapy was associated with longer progression free survival, longer OS, higher ORR and more complete responses than lenvatinib monotherapy (21). In addition, 9 patients in the LeToHAIC group received curative surgical resection following tumor shrinkage. A further study by Zhang et al. of 34 patients with unresectable liver cancer who were treated with PD-1 inhibitors combined with TKI and TACE, reported a conversion resection rate of 56% (20).

Transarterial Radioembolization With Yttrium-90 Microspheres

TARE with Y90 microspheres is a form of selective internal radiotherapy (31). Several studies have successfully demonstrated tumor downstaging with TARE in patients with unresectable HCC (11, 12, 32). In a review of 349 patients with unresectable HCC who received TARE, 48% of whom were Child-Pugh class C, 10 were subsequently able to undergo liver resection, and an additional 22 patients received a liver transplant (11). In this cohort, TARE was associated with a decrease in viable nodules and led to tumor downsizing and downstaging (based on BCLC staging criteria). A single-center study reported that around 20% of patients with unresectable HCC and portal vein thrombosis achieved downstaging following TARE and became eligible for surgery (12). Of the 24 patients included in this series, five received surgical resection following TARE, four underwent right trisectionectomy and one received a liver transplant. Median survival in the five patients was 54 months (95% confidence interval [CI]: 17-92) compared to median survivals of 30 months (95% CI: 18-42) and 11 months (95% CI: 8-14) in patients who achieved partial response/stable disease (n=8) and those with progressive disease (n=11) following TARE. In this series, high tumor absorbed radiation and low pre-treatment alpha-fetoprotein (AFP) levels were significantly associated with the probability of successful downstaging. A further similar analysis of 21 patients with United Network for Organ Sharing (UNOS) stage T3 HCC indicated that 6/21 patients were downstaged and eligible for radical treatment with curative intent following TACE (13). Of these six patients, three underwent resection, two received a liver transplant, and one received ablation and then underwent resection. In this series, patients who were treated radically were significantly younger and had higher tumor volumes than those who did not achieve radical treatment. Finally, a number of studies that assessed the role of selective internal radiation therapy (SIRT) as conversion therapy for unresectable liver cancer have been summarized in a literature review by Cucchetti and colleagues (33). These authors suggest that SIRT can lead to considerable downsizing of tumors, and also promote hypertrophy of the contralateral lobe. A complete response rate of approximately 10% in patients with HCC receiving SIRT and an objective response rate of ~40% were reported, coupled with a maximum contralateral hypertrophy above 40% (33).

There is evidence to suggest that TARE may offer advantages over TACE in terms of improved response rates and safety as a candidate modality for conversion therapy. In a non-randomized trial, TARE was associated with a higher response rate than TACE (partial response rates of 61% and 37%) and resulted in more patients being downstaged from UNOS T3 to UNOS T2 and becoming eligible for transplant (14). Almost all patients in this study had a Child-Pugh class of A (56% and 53%) or B (44% and 42%) across the TARE and TACE groups respectively. A retrospective analysis of patients treated with TACE or TARE over a 9-year period also indicated that TARE offered several advantages, including an improved response rate, longer time to progression, and less toxicity compared with TACE (18). In both the TARE and TACE groups most patients had a Child-Pugh class of A (54% and 55%) or B (44% and 43%). Zori and colleagues reported that TARE is associated with improved survival and less microvascular invasion, and required fewer administrations over the same time period compared with TACE (34). Finally, another advantage of TARE may be its ability to cause hypertrophy of the contralateral future liver remnant, which may be useful in potential candidates for resection with a small liver remnant (35–37).

Radiotherapy

The potential benefit of adding external beam radiotherapy (EBRT) to TACE as a strategy for tumor downstaging has been evaluated in multiple studies. A retrospective analysis of 203 patients with unresectable HCC without tumor thrombus, lymph node involvement, or extrahepatic metastases indicated a significant improvement in objective response rate among patients who received TACE plus EBRT compared with those who received TACE alone, and a numerical increase in the number of patients who became eligible for resection (38). Patients were selected for the combination therapy according to physician preference, and in most cases (49/54) this was due to defective lipiodol uptake during the TACE procedure, assessed by follow-up computed tomography (CT) scan. Approximately 85% of the patients enrolled in this study had tumors measuring >5 cm and were considered unsuitable for curative treatment. Objective response rates were 76% (41/54) among patients receiving EBRT plus TACE and 30.9% (46/149) among those receiving TACE alone (P<0.001), and sequential resection rates were 20.4% (11/54) compared with 12.8% (19/149), respectively (P=0.177) (38). Another retrospective study from the same group compared hepatic artery ligation plus radioimmunotherapy (RIT with 131I Hepama-1) with TACE plus EBRT in patients with unresectable HCC (15). Within the RIT group 4 patients (11%) had portal vein thrombi compared to 7 patients (20%) in the EBRT group. Objective responses were achieved in 85% (30/35) of those in the TACE plus EBRT group, and sequential resection rates following treatment were 23% (8/35) in this group (15). In addition, a randomized clinical trial evaluated the efficacy and safety of TACE plus external beam radiotherapy (TACE-RT group) compared with sorafenib for patients with hepatocellular carcinoma and macroscopic vascular invasion (39). The TACE-RT group showed a significantly higher radiologic response rate than the sorafenib group at 24 weeks (15 [33.3%] vs 1 [2.2%]; P < .001), and curative surgical resection was conducted for 5 patients (11.1%) in the TACE-RT group owing to downstaging.

Finally, one study evaluated the oncological outcomes and prognostic factors of surgical resection after downstaging with localized concurrent chemoradiotherapy (CCRT) followed by hepatic arterial infusion chemotherapy (HAIC) in HCC patients with portal vein tumor thrombosis (PVTT) (40). Among 98 patients in the CCRT group, 26 patients (26.5%) underwent subsequent curative resection. During the same study period, 18 patients with PVTT underwent surgical resection as the first treatment. Clinicopathological characteristics and oncological outcomes between groups were compared. The median follow-up period was 13 months (range 1-131 months). Disease-specific survival was significantly different between the resection after localized CCRT group and the resection-first group (40). Overall, these studies indicate that combination regimen based on radiotherapy may represent a useful adjunctive treatment for patients with potentially resectable HCC who are candidates for tumor downstaging.

SYSTEMIC THERAPY

Systemic therapies including tyrosine kinase inhibitors (TKIs), immunotherapy, and chemotherapy, are widely used in the palliative treatment of HCC. Traditionally, systemic therapies for HCC have been associated with relatively low response rates and therefore neoadjuvant and downstaging therapy have not been part of standard management protocols. However, recent advances in systemic therapy have seen improved response rates, leading to a re-evaluation of the value of systemic therapy in the conversion therapy setting (**Table 2**).

Several studies have reported high ORRs with lenvatinib, either alone or in combination with other systemic treatments, in patients with initially unresectable HCC, making this a promising option for conversion/downstaging therapy. In the phase 3 REFLECT trial, lenvatinib was associated with a significantly higher ORR than sorafenib in patients with advanced unresectable HCC who had not received prior treatment for advanced disease and were Child-Pugh class A (41). In this non-inferiority study, the ORR was 40.6% in patients receiving lenvatinib versus 12.4% in those treated with sorafenib (odds ratio [OR], 5.01 [95% CI: 3.59-7.01]; P<0.0001) by modified RECIST (mRECIST), and the ORR by RECIST was 18.8% versus 6.5% (OR, 3.34 [95% CI: 2.17-5.14]; P<0.001). A network meta-analysis compare response rates, survival outcomes, and safety of first-line systemic therapies for advanced hepatocellular carcinoma, and the results showed that lenvatinib is associated with the best ORR of all systemic therapies included in the analysis (67). A further study from Japan included 107 consecutive patients who underwent lenvatinib treatment for advanced HCC, the majority of

TABLE 2 | Summary of efficacy data of systematic treatments for advanced HCC.

Line		Treatment regimen	Study name	n	OS (months)	PFS (months)	ORR[a] (%)	TTR (months)	Grade ≥3 TRAE (%)
1L	Mono	Lenvatinib (Global) (41)	REFLECT	478	13.6	7.3	18.3	–	57.0
		Lenvatinib (China) (42)	REFLECT	144	15.0	9.2	21.5[b]	–	44.0
		Sorafenib (43)	ORIENTAL	150	6.5	–	3.3	–	47.7[d]
		Sorafenib (44)	SHARP	299	10.7	–	2.0	–	52.0[f]
		FOLFOX4 (45)	EACH	184	6.4	2.94	8.15	–	55.7[c]
		Donafenib (46)	ZGDH3	328	12.0	3.7	4.6	–	37.5
	Combo	Lenvatinib+nivolumab (47)	Study117	30	–	7.39[b]	54.2	–	60.0[c]
		Lenvatinib+pembrolizumab (48)	Keynote524	100	22.0	8.6	36.0	2.8	67.0
		Apatinib+camrelizumab (49)	RESCUE	70	20.3	5.7	34.3	1.9	77.4
		Bevacizumab+atezolizumab (50, 51)	IMbrave150	336	19.2	6.9	30.0	–	43.0
		Regorafenib+pembrolizumab (52)	–	22	–	–	29.0	–	86.0[c]
		Anlotinib+penpulimab (53)	–	31	–	–	31.0	–	19.4
		Sintilimab+IBI305 (54)	ORIENT 32	380	–	4.6	21.0	–	56.0[c]
		Lenvatinib+AK104 (55)	–	18	–	–	44.4	–	26.7
		Lenvatinib+CS1003 (56)	–	20	–	8.4	40.0	–	35.0[c]
		Avelumab+axitinib (57)	VEGF Liver 100	22	14.1	5.5	13.6	–	72.7
2L	Mono	Pembrolizumab (58)	KEYNOTE240	278	13.9	3.0	18.3	–	18.6
		Regorafenib (59)	RESORCE	379	10.6	3.4	6.5	–	50.0
		Cabozantinib (60)	CELESTIAL	470	10.2	5.2	3.8	–	67.7[e]
		Camrelizumab (61)	–	217	13.8	2.1	14.7	2.0	22
		Apatinib (62)	AHELP	261	8.7	4.5	10.7	–	77.4
		Tislelizumab (63)	RATIONALE208	249	13.2	2.7	13.3	–	14.5
	Combo	Cabozantinib+nivolumab+ipilimumab (64)	–	35	NR	6.8	29.0	–	71.0
		Cabozantinib+nivolumab (64)	–	36	21.5	5.40	19.0	–	47.0
		Nivolumab+ipilimumab (65)	–	50	22.8	–	32.0	–	53.0
		Durvalumab+tremelimumab (66)	Study 22	74	18.7	2.17	24.0	–	35.1

[a]*According to RECIST v1.1.*
[b]*According to mRECIST.*
[c]*TEAE.*
[d]*Treatment-emergent serious adverse events (SAE).*
[e]*AE.*
[f]*SAE.*

patients in this study had an ECOG score of 0 (87.9%) and were Child-Pugh class A (92.5%). Of the 107 patients included, 16 subsequently received surgical intervention, and R0 resection was achieved in nine (8.4%) patients. Survival analysis confirmed that successful conversion to R0 resection was associated with a longer time to treatment failure (68). Thus, data from the studies suggest that lenvatinib may have utility as a conversion therapy in patients who present with advanced, initially unresectable HCC.

Results from several small sample studies also support combination therapy with TKIs combined with immunotherapy in the conversion treatment setting in patients with unresectable HCC. For example, Zhu et al. reported 63 cases of patients with initially unresectable liver cancer treated with PD-1 inhibitors combined with a TKI, 60 of whom were classed as Child-Pugh A, and the conversion resection rate was 15.9% (19). A further study by Lu et al. of 33 patients with unresectable liver cancer with measurable PVTT (by mRECIST) and no extrahepatic metastasis who were treated with PD-1 inhibitors combined with Lenvatinib, reported a conversion rate of 42.4% (22). Huang reported that the response of intrahepatic tumor was less significant than that of tumor thrombi when treated with the combination of lenvatinib and PD-1 antibody (69), which suggested locoregional therapy can be used to improve the control of intrahepatic tumor when tumor thrombi is necrotic.

MULTIMODAL TREATMENT APPROACHES

Multimodality treatment approaches combine two or more treatment modalities with the aim of improving clinical outcomes beyond those achieved with either modality alone. Combining multiple therapies may provide cumulative benefits in term of efficacy beyond those offered by either modality alone, but may also be limited by non-overlapping toxicity profiles inherited from both modalities. Studies on multimodality treatment approaches in the setting of HCC conversion therapy with the intent of HCC downstaging are currently limited.

Concurrent chemoradiotherapy has been assessed for downstaging patients with unresectable HCC (16). In this study, 264 patients received radiotherapy (45.0 Gy with fractional dose of 1.8 Gy) and concurrent intra-arterial chemotherapy with 5-fluorouracil (500 mg/day), 15 of whom (83.3%) were classified as Child-Pugh class A. Eighteen of these patients (6.8%) subsequently underwent hepatic resection after achieving a response,. At the time of surgery, six patients had complete response, 11 had partial remission, and one had stable disease. In this study, cases were considered resectable when tumor-free margins and sufficient remnant volumes were obtained without extrahepatic metastasis. Median time from chemoradiotherapy to resection was 6.2 months (range 1-21 months), and median OS and disease-free survival were 61.8 months and 24.1 months, respectively. Three patients remained without evidence of disease recurrence for >5 years and all three had complete or partial remission with 90-100% necrosis post surgery (16).

A network meta-analysis of different embolization treatment strategies for unresectable HCC suggested that chemoembolization combined with external radiotherapy or local liver ablation could significantly improve tumor response rates compared with embolization alone (70). The OR (odds ratio) for achieving an objective response relative to control was 142 (95% CI: 55.9-395.4) with TACE plus ablation and 13.9 (95% CI: 6.9-31.3) with TACE alone. However, all treatments assessed in this analysis also increased the risk for serious adverse events relative to control, with the largest increases seen in patients receiving two treatment modalities. For example, the OR for a serious adverse event relative to control in patients receiving TACE plus ablation was 11.7 (95% CI: 1.5-128.7), and 14.6 (95% CI: 4.7-67.7) in those receiving TACE alone (70).

DISCUSSION

Surgery remains the only potentially curative treatment modality for patients with resectable HCC and normal liver function; however, only a minority of patients with HCC are eligible for resection at diagnosis. Conversion therapy aimed at tumor downstaging can increase the proportion of patients with HCC who are eligible for surgical resection; however, this approach is not routinely recommended in clinical practice at present due to a lack of supporting evidence. Despite this, accumulating evidence suggests that selected patients may achieve adequate downstaging following TACE or TARE to enable surgical resection. Lenvatinib-based combination therapy is also a promising option for conversion therapy in patients with potentially resectable disease with encouraging ORRs reported in clinical trials enrolling patients with initially unresectable HCC. Multimodality therapy may increase the proportion of patients eligible to undergo surgery or other curative treatments and reduce disease recurrence rates, allowing patients to experience a cancer-free, and drug-free status with long survival and good quality of life (71). It should be mentioned that there are other non-tumor focused approaches to conversion therapy such as techniques aiming to increase residual liver volume by inducing liver hypertrophy such as portal vein ligation (PVS) (72–74), artery ligation (75), and associating liver partition and portal vein ligation for staged hepatectomy (ALPPS) (76, 77). However, limitations of these approaches include a relatively long time for liver hyperplasia following PVE, which increases the chance of further disease progression, and an increased risk of complications with the use of ALPPS (78).

Ultimately, data from large randomized controlled trials are still required to clarify the clinical benefit of conversion therapy in patients diagnosed with unresectable HCC and identify specific patient groups likely to benefit from this approach. There is also a need for clearer definitions regarding which patients should initiate downstaging protocols and what criteria should be met before resection can be attempted. This will become increasingly important if treatments aimed at

downstaging begin to differ from those used for palliative care. For now, preliminary data support the concept of downstaging in patients with potentially resectable HCC, offering the potential to provide clinical benefit to a population of patients in urgent need of expanded treatment options.

AUTHOR CONTRIBUTIONS

H-CS and X-DZ contributed to the conception and conduct of this review article, drafted and revised the article. All authors contributed to the article and approved the submitted version.

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Downstaging Therapies for Unresectable Hepatocellular Carcinoma Prior to Hepatic Resection: A Systematic Review and Meta-Analysis

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Introduction: Hepatocellular carcinoma (HCC) is a high-grade malignant disease with unfavorable prognosis, and although surgical therapy is necessary, not all patients with HCC are suitable candidates for surgery. Downstaging as preoperative therapeutic strategy, which can convert unresectable HCC into resectable HCC, intends to increase the resection rate and improve prognosis.

Methods: We searched multiple databases updated to December 30, 2020, for studies on transcatheter arterial chemoembolization (TACE), Yttrium 90 microsphere selective internal radiation (SIR)/transcatheter radioembolization (TARE), hepatic arterial infusion (HAI), and systemic treatment as downstaging treatment before resection for patients with unresectable HCC.

Results: A total of 20 comparative and non-comparative studies were finally included in the meta-analysis. The pooled downstaging rate of hepatic resection (HR) was 14% [95% confidence interval (CI) 0.10–0.17] with significant heterogeneity (I^2 = 94.51%). The chemotherapy, combination, and non-cirrhosis groups exhibit higher rates of downstaging, but these differences were not significant. For comparative studies, the overall survival (OS) rates of resection after downstaging were far better than those inpatients who received locoregional therapy (LRT) or systemic treatment alone at 1 year (RR 1.87, 95% CI 1.48–2.38), 3 years (RR 5.56, 95% CI 2.55–12.10), and 5 years (RR 5.47, 95% CI 2.22–13.49). In addition, the pooled disease-free survival (DFS) rates in patients undergoing HR after successful downstaging were 78% (95% CI 0.62–0.93) at 1

year, 47% (95% CI 0.25–0.68) at 3 years, and 46% (95% CI 0.32–0.59) at 5 years. The pooled OS rates were 88% (95% CI 0.82–0.95) at 1 year, 64% (95% CI 0.59–0.69) at 3 years, and 42% (95% CI 0.29–0.54) at 5 years.

Conclusions: Downstaging may serve as a screening tool to identify patients who might benefit from surgery. Resection after successful downstaging can improve prognosis.

Keywords: hepatic resection (HR), downstaging, hepatocellular carcinoma, unresectable, meta-analysis

INTRODUCTION

Hepatocellular carcinoma (HCC) is a malignant disease that ranks sixth in morbidity and fourth in fatality among cancers globally (1). Hepatic resection (HR) and liver transplantation (LT) are the main curative-intent options, which offer 5-year survival rate exceeding 70% in patients with early HCC. However, the application of LT is limited by a shortage of donors. Thus, HR is currently a popular curative therapy. The indications for HR in treating HCC remain controversial. According to guidelines from the European Association for the Study of the Liver (EASL) (2), American Association for the Study of Liver Disease (AASLD) (3), and National Comprehensive Cancer Network (NCCN) (4), following the Barcelona Clinic Liver Cancer (BCLC) system (5), only patients with stage A are resectable. Unfortunately, based on the indication, most patients miss the time window for surgical therapy, leaving less than 30% of patients' resectability at the time of diagnosis.

In this context, the indications for HR extend beyond the early stage of HCC in clinical practice (6–8). The Asian Pacific Association for the Study of the Liver (APASL) guidelines set wider indications for HR (9); some patients with BCLC stages B–C can be considered candidates for resection in terms of tumor burden and liver functional reserves. Moreover, comprehensive strategies are required to create opportunities for resection. Therapies that include locoregional therapies (LRTs) such as transcatheter arterial chemoembolization (TACE), Yttrium 90 microsphere selective internal radiation (SIR)/transcatheter radioembolization (TARE), hepatic arterial infusion (HAI) for patients with stage B, and systemic treatment such as molecular targeting and chemotherapy for patients with stage C aim at reducing the tumor load and tumor stage to convert unresectable HCC to resectable HCC, or to make it easier to remove the tumor radically; this approach is referred to as downstaging (10). Downstaging strategies are recommended for patients exceeding the Milan criteria considered for LT by the EASL, AASLD, and NCCN (2–4). However, since most studies on downstaging of HR have had small sample sizes, inconsistent inclusion criteria, great difference in results, and lack of prospective clinical trials with large samples, no consensus has been reached yet on HR. There are no systematic reviews or meta-analyses of downstaging prior to HR in patients with advanced unresectable HCC. Therefore, we systematically summarized studies on downstaging therapies for HCC, to synthesize the existing evidence regarding the efficacy of LRT or systemic therapies as downstaging strategies for patients with unresectable HCC who are potential candidates for resection.

MATERIALS AND METHODS

All methods were performed according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) (11) and MOOSE (Meta-analysis of Observational Studies in Epidemiology) (12) reporting guidelines.

Literature Screening and Search Strategy

We thoroughly searched all relevant studies updated to December 30, 2020, in the PubMed (https://www.ncbi.nlm.nih.gov/pubmed), Cochrane (https://www.cochranelibrary.com), Embase (https://www.embase.com), Web of Science (https://www.webofknowledge.com), VIP (http://www.cqvip.com), Wanfang (http://www.wanfangdata.com.cn), and CNKI (https://www.cnki.net) database. The search strategy was as follows: [(hepatocellular carcinoma) OR (hepatocellular cancer) OR (liver cancer) OR (hepatic neoplasm)] AND [(downstaging) OR (downstage) OR (down stage) OR (conversion therapy) OR (preoperative treatment) OR (preoperative treatments) OR (preoperative therapy) OR (preoperative therapies)] AND [(hepatic resection) OR (hepatectomy) OR (salvage)].

Study Selection

Two authors independently conducted the literature search and initially selected relevant studies by reading titles and abstracts. Studies describing irrelevant subjects were excluded from the first step. Furthermore, the remaining studies were further screened by reading the full texts, and ineligible studies were discarded. Our inclusion criteria were as follows: (1) patients who were diagnosed as unresectable HCC, including extrahepatic disease or extensive local disease not amenable to definitive resection; (2) intervention included any treatment for HCC that reduced the tumor load and tumor stage (e.g., TACE, radiofrequency ablation, SIR/TARE, systemic therapy, or a combination of therapies); (3) studies eligible for our meta-analysis included prospective and retrospective comparative studies, cohort observational studies, and case series; and (4) outcomes evaluating rates of success for downstaging, overall survival (OS), disease-free survival (DFS) rate, and recurrence-free survival (RFS) rate. We excluded articles that (1) included less than five patients; (2) reported duplicate cohorts of patients, in which case we used the most updated cohort or the

most recent publication; (3) were conference proceedings, letters, literature reviews, systematic reviews, case reports, comments, animal experiments, or unpublished studies with no full-text availability; (4) applied no restrictions to the language of articles; (5) reported downstaging used in LT, unless the studies also included a resection group, the data of which could be used to evaluate the downstaging success rate or survival rate could be extracted separately.

Data Extraction

For each study, data were extracted in duplicate using standardized forms. We extracted the following variables from each study: study characteristics (first author's last name, publication year, study design, and sample size), downstaging treatment modality, downstaging rate, OS rate, and DFS rate. The outcomes we extracted from each study were as follows: (1) the success rate of downstaging to remove tumor completely and (2) the long-term survival for HR with downstaging.

Quality Assessment

The quality of randomized controlled trials (RCTs) and non-randomized controlled trials (nRCTs) were assessed using the Cochrane bias assessment tool (13) and methodological index for non-randomized studies (MINORS) (14), respectively. The observational study quality was based on the modified Newcastle-Ottawa scale (NOS) (15). The Institute of Health Economics Quality Appraisal (IHEQA) Checklist (16) was used to assess methodological quality for case series studies without a control group.

Statistical Analysis

For comparative studies, we calculated the relative risks (RRs) and 95% confidence intervals (CIs) employing a binomial distribution. For noncomparative studies, we calculated the event rates of outcomes, and we estimated 95% CIs using Jeffreys method. A heterogeneity test for homogeneity of effect size was also given. Heterogeneity was assessed by I^2 statistics and the p-value of the Chi-square test (17). A random-effects model was used to merge the data and estimate effect-size indicators according to the Cochrane Handbook for Systematic Reviews of Interventions version 6.2 (18). Statistical analysis was conducted using Stata 15.0 software (Stata Corporation, College Station, TX, USA), and a p-value <0.05 was considered statistically significant for the effect size of each included study.

RESULTS

Study Characteristics and Quality Assessment

The flowchart of the study selection process is shown in **Figure 1**. After searching seven electronic databases, 3,348 citations were retrieved initially, of which 310 for screening titles and abstracts and 66 full-text for reviewing. A total of 20 studies were finally included in the meta-analysis (**Table 1**). The quality assessment of the included studies is displayed in **Supplementary Tables 1–3**. Two prospective single-arm studies had a moderate risk of bias, and six observational studies received NOS scores of ≥7, while 12 case series had a high risk of bias. In total, most inclusive studies were rated as low-moderate quality. There were no RCT identified.

Downstaging Rate

There were 17 studies (4 comparative studies and 13 non-comparative studies) covering 20 subgroups with 4,878 patients that evaluated downstaging rate. There was only one case–control study comparing two chemotherapy regimens of downstaging; therefore, a single-arm meta-analysis was conducted for the downstaging success rate (**Figure 2A**). The pooled downstaging rate of HR was 14% (95% CI 0.10–0.17) with significant heterogeneity (I^2 = 94.51%). Subgroup analyses were performed by intervention, mono/multitherapy, and if patients with cirrhosis or extrahepatic spread were included. In the intervention group, data were sufficient to perform subgroup analysis on TACE and chemotherapy; the downstaging success rate displayed nonsignificant reduction in the TACE group (I^2 = 41.9%) compared to that in the chemotherapy group (I^2 = 80.98%) (20% *vs.* 14%; p = 0.226; **Figure 2B**), and the statistical heterogeneity decreased. Meanwhile, the downstaging rate of combination therapy (I^2 = 96.61%) demonstrated a non-significant trend towards improvement over monotherapy (I^2 = 92.05%) nonsignificantly (17% *vs.* 12%, p = 0.338; **Figure 2C**). Studies that included patients with cirrhosis (I^2 = 88.13%) had a lower success rate of downstaging than studies that did not, although the difference was not significant (I^2 = NA) (13% *vs.* 17%, p = 0.266; **Figure 2D**). In addition, extrahepatic spread did not exhibit a link with downstaging rate (I^2 = 83.05%) (14% *vs.* 11%, p = 0.273; **Supplementary Figure 1**).

DFS Rate

Four non-comparative studies with 142 patients investigated the DFS or RFS rate (referred to later in this paper as DFS). Three articles reported 1-year DFS rate; four reported 3-year DFS rates, and two reported 5-year DFS rates. The 1-year DFS rate was 78% (95% CI 0.62–0.93, I^2 = NA), 3-year DFS rate was 47% (95% CI 0.25–0.68, I^2 = 79.25%), and the 5-year DFS rate was 46% (95% CI 0.32–0.59, I^2 = NA) (**Figure 3**).

OS Rate

Four comparative studies enrolled 492 patients documented data for OS rates (**Figure 4**). Compared with non-surgical treatment, HR after downstaging therapy showed a significant increase in the 1-year OS rate (RR 1.87, 95% CI 1.48–2.38, I^2 = 0.0%), 2-year OS rate (RR 2.44, 95% CI 1.06–5.59, I^2 = 90.6%), 3-year OS rate (RR 5.56, 95CI 2.55–12.10, I^2 = 42.7%), 4-year OS rate (RR 5.56, 95% CI 2.55–12.10, I^2 = 42.7%), and 5-year OS rate (RR 5.47, 95% CI 2.22–13.49, I^2 = 61.4%). Twelve non-comparative studies investigated the OS rate in 618 patients in 17 subgroups (**Figure 5A**). Eight articles reported 1-year OS rates, 10 reported 3-year OS rates, and 9 reported 5-year OS rates for 10 subgroups. The 1-year pooled OS rate (I^2 = 73.54%) was 88% (95% CI 0.82–0.95), the 3-year pooled OS rate (I^2 = 6.58%) was 64% (95% CI 0.59–0.69), and the 5-year combined OS rate (I^2 = 87.13%) was 42% (95% CI 0.29–0.54). Subgroup analysis showed that the 3-year OS rate of extrahepatic

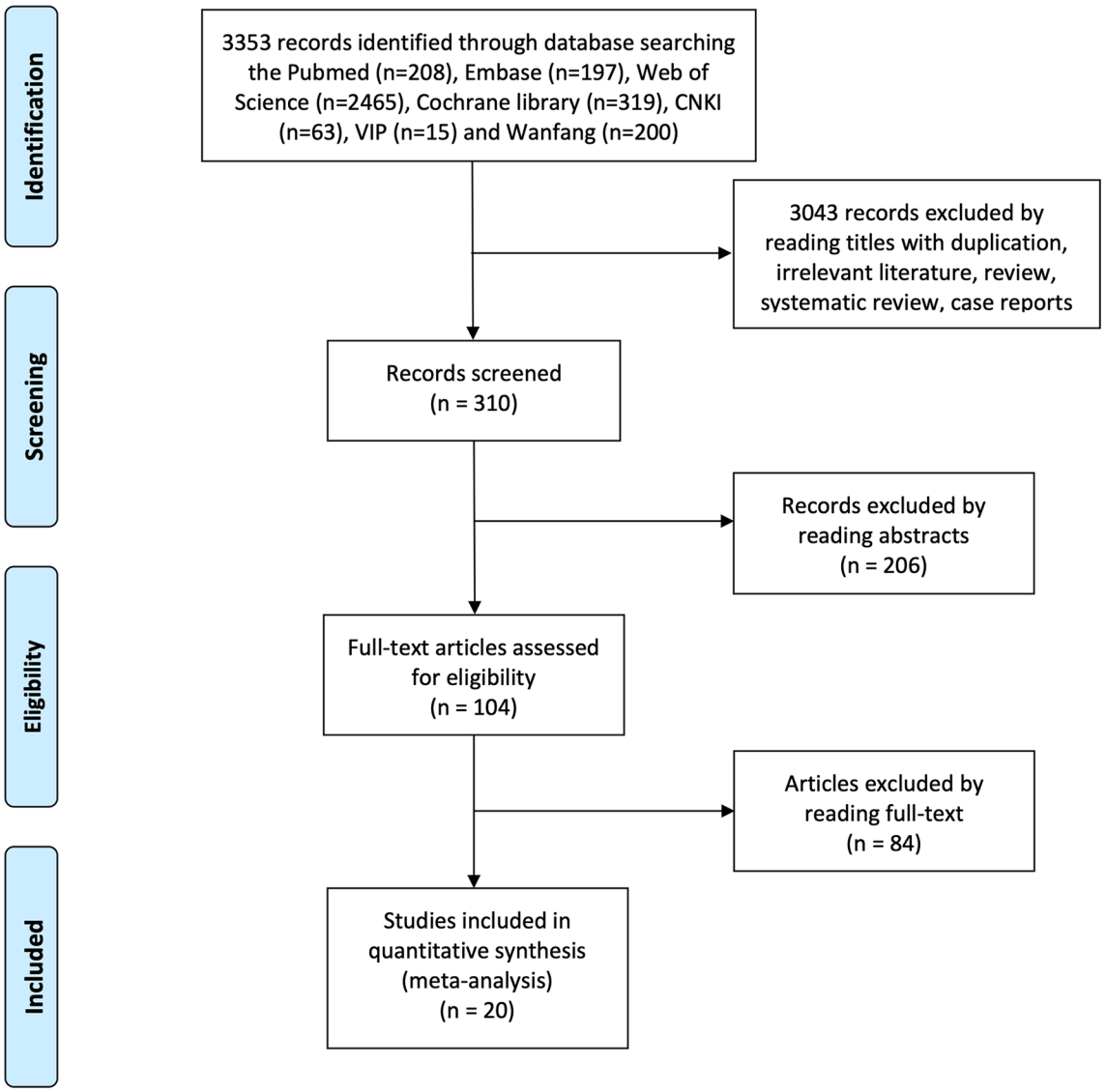

FIGURE 1 | Flowchart illustrating the study selection of downstaging prior to HR.

disease was higher than that of the subgroup without extrahepatic disease, but the difference was not statistically significant. The 5-year OS rate in patients with extrahepatic disease was comparable to that in patients without extrahepatic disease (**Figures 5C, D**). Subgroup analysis by modality for the 5-year OS rate revealed that the efficacy of multitherapy was better than that of monotherapy (0.26 *vs.* 0.06, $p = 0.02$) (**Figure 5B**).

DISCUSSION

Unresectable HCC is generally considered incurable. However, the definition of resectable/unresectable is subjective in accordance with the extent of tumor, functional liver reserve, and surgeons' judgments. Although conversion surgery may be applied following adequate downstaging achieved by tumor downsizing methods or increasing the future liver remnant (FLR), a common criticism is that technically resectable does not represent the optimal oncological outcome. Thus, we conducted this meta-analysis to determine whether downstaging therapies aimed at shrinking tumors are feasible or effective for unresectable intermediate and advanced HCC.

Our meta-analysis is the first to synthesize the existing evidence on the success rates and effectiveness of LRT and/or systemic treatment as downstaging strategies prior to HR in patients with advanced unresectable HCC, and it confirms that only approximately 14% of patients attain downstaging after initiation of HR. The chemotherapy, combination, and non-cirrhosis groups exhibited higher rates of downstaging, but these differences were not

TABLE 1 | Studies of downstaging therapy for hepatic resection of HCC.

Study	Year	Intervention	Types of intervention	N of receiving downstaging	Design	Reason of unresectability
Sitzmann (19)	1993	Combined RT and CT	LRT+systemic treatment	14	Retrospective cohort	Extrahepatic metastasis; diffuse liver tumor or major vascular invasion
Majno (20)	1997	TACE	LRT	49	Case series	Three or more intrahepatic tumor nodules
Fan (21)	1998	TACE	LRT	65	Case series	Too bulky for resection or situated centrally at the hepatic hilus
Lau2 (22)	2001	Chemoimmunotherapy	Systemic treatment	150	Case series	Extrahepatic metastasis; diffuse liver tumor or major vascular invasion
Clavien (23)	2002	CT	Systemic treatment	28*	Prospective pilot study	Diffuse liver tumor; large solitary tumor or major vascular invasion
Lau1 (24)	2004	SIR/CT	LRT/systemic treatment	71 (SIR) 124 (PAIF) 75 (doxorubicin)	Case series	Extrahepatic metastasis; diffuse liver tumor or major vascular invasion
Tang (25)	2004	Multimodality	LRT	379 (HAI) 1085 (HAI+HAL) 562 (HAI+HAL +RAIT)	Case series	NA
Zhao (26)	2009	TACE or TACE+PEI or TACE-RT	LRT	34	Case series	Too bulky for resection; diffuse liver tumor or major vascular invasion
Shi (27)	2012	TACE	LRT	412	Case series	Too bulky for resection or located centrally at the hepatic hilus
Chen (28)	2013	TACE	LRT	433	Case series	NA
Kaseb (29)	2013	CT (mPAIF *vs*. PAIF)	Systemic treatment	117 (33 *vs*. 84)	Retrospective cohort	Extrahepatic metastasis; diffuse liver tumor or major vascular invasion
Lee1 (30)	2014	HAI+CCRT followed by resection *vs*. HAI+CCRT alone	LRT	243 (41 *vs*. 202)	Retrospective cohort	Too bulky for resection; diffuse liver tumor or major vascular invasion
Lee2 (31)	2014	CCRT	LRT	41	Retrospective cohort	Too bulky for resection; major vascular invasion
Zhang (32)	2016	TACE-RT	LRT	82 (43 *vs*. 39)	Retrospective cohort	NA
Li (33)	2017	TACE+sorafinib	LRT+systemic treatment	21	Case series	BCLC stage B-C
Hamaoka (34)	2017	HAI+RT followed by resection *vs*. HAI +RT alone	LRT	50 (7 *vs*. 43)	Retrospective cohort	HCC with PVTT
He (35)	2018	HAI+sorafenib	LRT+systemic treatment	35	Prospective single-arm	Extrahepatic metastasis; HCC with PVTT
Lee3 (36)	2019	HAI	LRT	103	Case series	Extrahepatic metastasis; diffuse liver tumor or major vascular invasion
Goto (37)	2020	HAI	LRT	18	Retrospective cohort	Diffuse liver tumor or major vascular invasion
Chiu (38)	2020	DEB-TACE *vs*. cTACE	LRT	61(42 *vs*. 19)	Retrospective cohort	Diffuse liver tumor or major vascular invasion

N, number of patients; NA, not available; RT, radiotherapy; CT, chemotherapy; TACE, transarterial chemoembolization; SIR, selective internal radiation; PAIF, cisplatin, doxorubicin, 5-fluorouracil, and interferon-alpha; HAI, hepatic arterial infusion; PEI, percutaneous ethanol injection; RAIT, radioimmunotherapy; mPAIF, modified PAIF; BCLC, Barcelona Clinic Liver Cancer; DEB-TACE, drug-eluting beads transarterial chemoembolization; cTACE, conventional transarterial chemoembolization.
**5 HCC and 23 metastatic colorectal cancer.*

significant. The conversion rate of HR is much lower than that of LT (39), which remains unsatisfactory. This might be explained by the fact that studies on downstaging for HR were fewer in number and of poorer quality compared to studies of LT, which often included LRT as a bridge to transplantation, and some studies enrolled patients who failed previous treatment. There is significant heterogeneity in terms of different inclusion criteria of downstaging, subjective judgment of resectability, and the selection of LRT, which is closely related to the experiences and preferences of each institution. In comparative studies, the OS rates of resection after downstaging were notably better than those in patients receiving LRT or systemic treatment alone at 1 year (RR 1.87, 95% CI 1.48–2.38), 3 years (RR 5.56, 95% CI 2.55–12.10), and 5 years (RR 5.47, 95% CI 2.22–13.49). In comparative studies, the pooled DFS rates of patients undergoing HR after successful downstaging were 78% (95% CI 0.62–0.93) at 1 year, 47% (95% CI 0.25–0.68) at 3 years, and 46% (95% CI 0.32–0.59) at 5 years. The pooled OS rates were 88% (95% CI 0.82–0.95) at 1 year, 64% (95% CI 0.59–0.69) at 3 years, and 42% (95% CI 0.29–0.54) at 5 years. These results were better than the reported data of non-surgical interventions (40), and worse than the industry-accepted survival rates of early HCC (4). As a result, downstaging may be considered an alternative strategy for patients with unresectable HCC. In this paper, downstaging is defined as systemic therapy or regional antitumor therapy with the aim of converting unresectable HCC into resectable HCC. It is necessary to optimize the downstaging strategies to further improve the effect from an intent-to-treat viewpoint. Although many studies have investigated the clinical

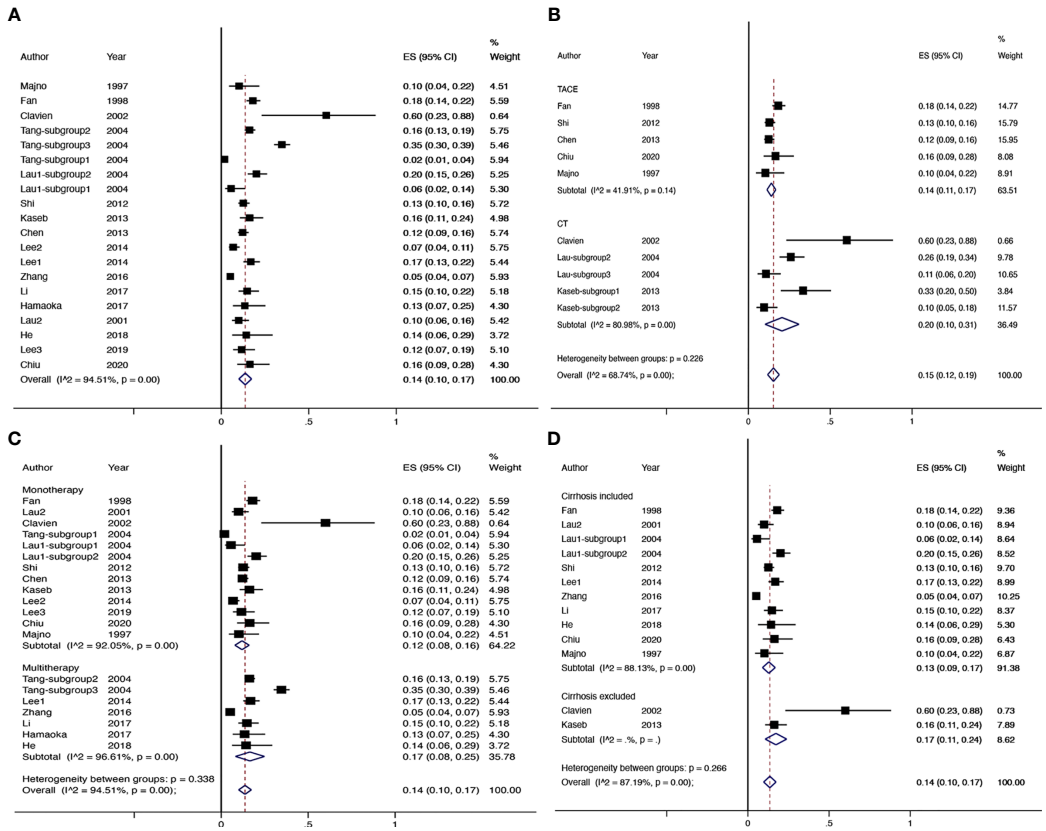

FIGURE 2 | The overall downstaging success rate of hepatic resection of HCC **(A)**, pooled downstaging rate stratified by TACE and CT **(B)**, pooled downstaging rate stratified by mono/multitherapy **(C)**; pooled downstaging rate stratified by cirrhosis included/excluded **(D)**. TACE, transarterial chemoembolization; CT, chemotherapy.

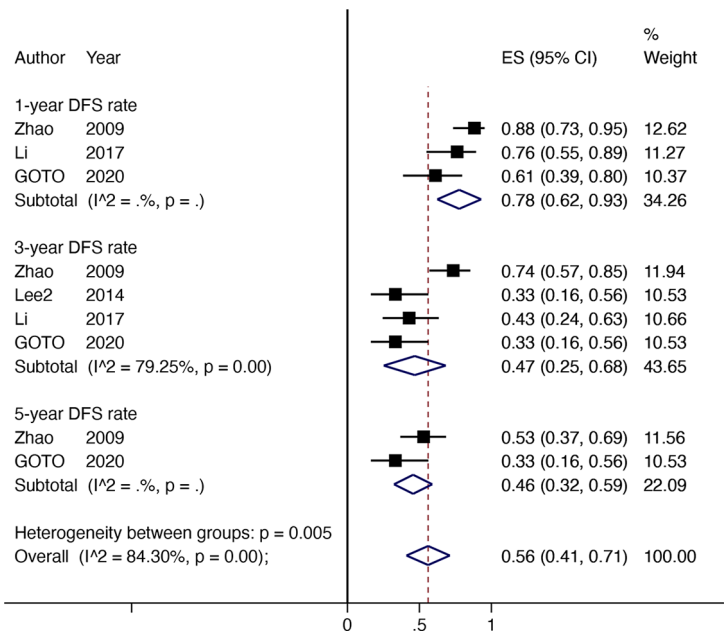

FIGURE 3 | The DFS rate for HR with downstaging therapies in patients with HCC.

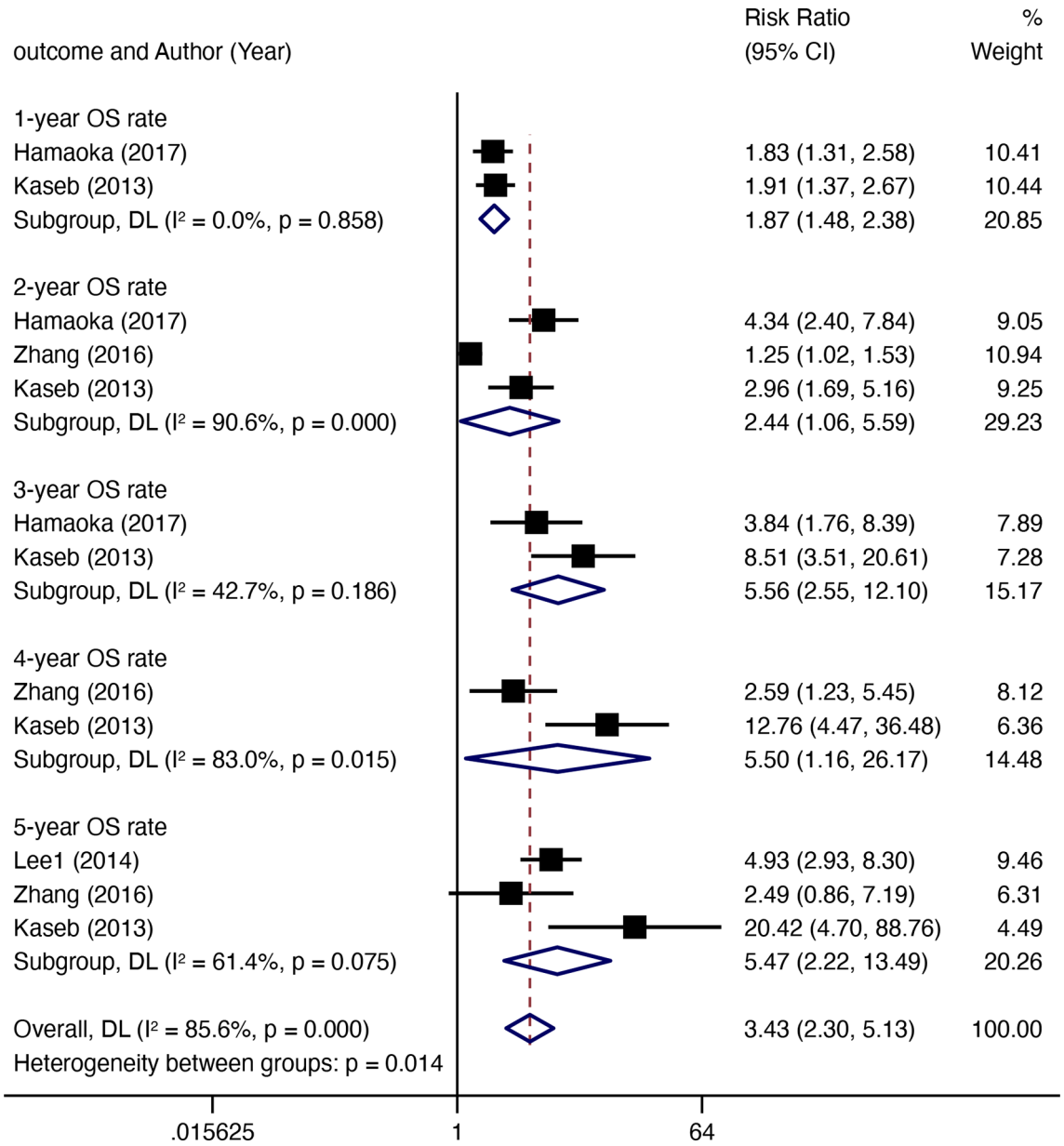

FIGURE 4 | The OS rate (resection after downstaging versus LRT or systematic treatment alone) for HR with downstaging therapies in patients with HCC.

efficacy of LRT or systemic therapy alone for unresectable HCC, the best intervention for downstaging therapy is not yet clear. The causes of unresectability varied in different studies, mainly including local extension, major vascular invasion, and extrahepatic spread. LRTs such as TACE, SIR, and HAI tend to be adopted in patients with local extension and major vascular invasion. TACE is considered a standard care for unresectable HCC and has also been widely used in downstaging strategies. Recently, TARE/SIR, as an alternative to TACE, has received increasing attention because it can effectively shrink a tumor and shorten the response time, and may be adopted for the patients of portal thrombosis (a contraindication of TACE) (41). Microwave and radiofrequency ablation have been used in LT downstaging, but there are no related reports on HR downstaging. Six articles reported patients with extrahepatic metastases, mainly using systemic treatment. LRT was only employed in one study, which showed that extrahepatic metastasis was not a significant factor in survival for patients with HAI with chemotherapy (36). The use of HAI with chemotherapy in HCC patients with minimal extrahepatic metastasis was supported in previous studies, for intrahepatic lesions have a greater impact on survival than extrahepatic lesions (42–44). Based on our meta-analysis, extrahepatic metastasis was not

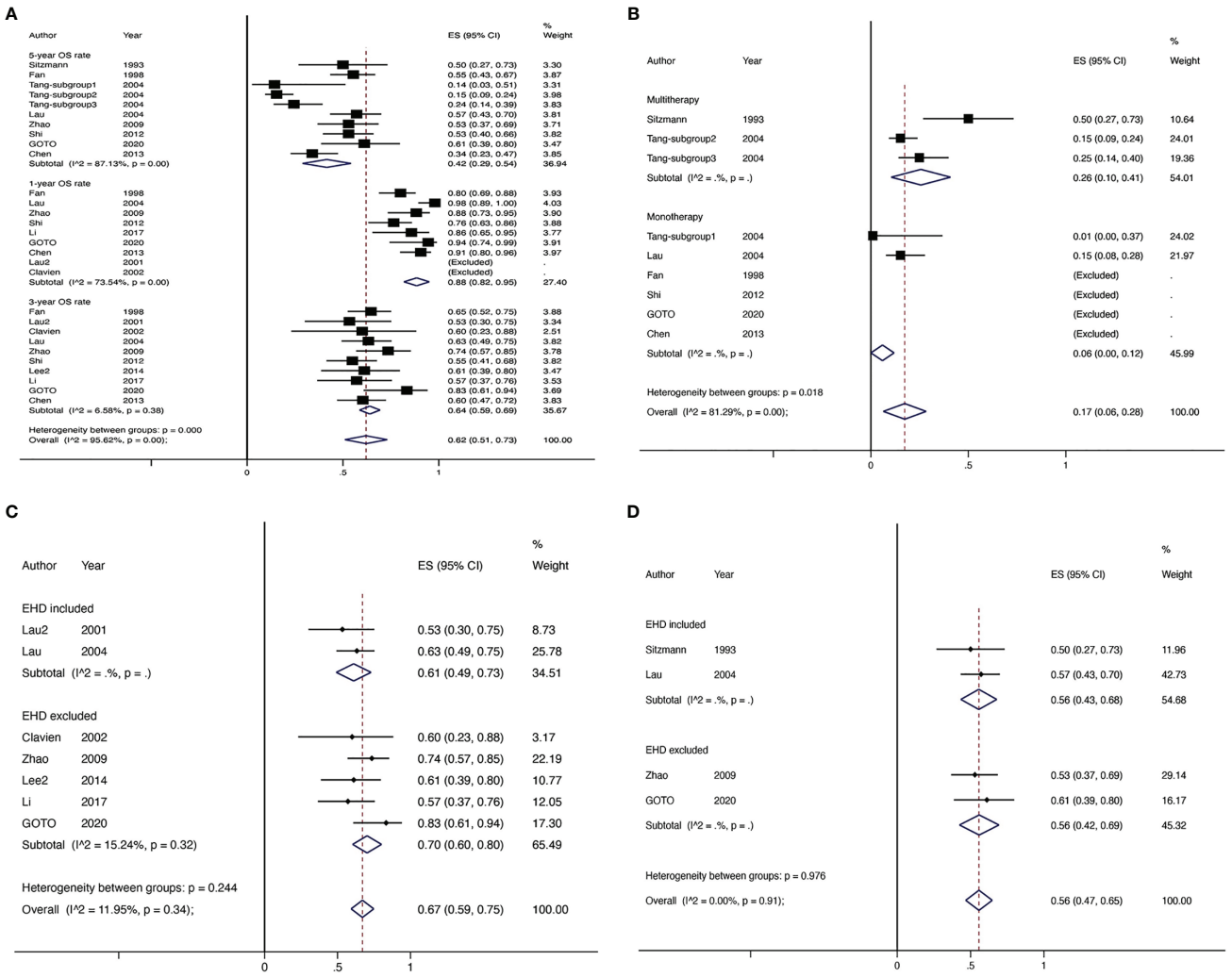

FIGURE 5 | The OS rate of non-comparative studies for HR with downstaging therapies in patients with HCC **(A)**, the 5-year OS rate of non-comparative studies for HR with downstaging therapies stratified by mono/multitherapy **(B)**, the 3-year OS rate of non-comparative studies for HR with downstaging therapies stratified by EHD included/excluded **(C)**, the 5-year OS rate of non-comparative studies for HR with downstaging therapies stratified by EHD included/excluded **(D)**. EHD, extrahepatic disease.

associated with the 3- and 5-year OS rate. Furthermore, the subgroup analyses of the downstaging rate on intervention and patients with or without cirrhosis showed no significant difference in the downstaging rate, while these data were insufficient for subgroup analysis on survival. Notably, the subgroup analysis of mono/multitherapy showed that multimodality downstaging prolongs long-term survival. This suggests that the results may not be affected by the selection of downstaging therapy, but more influenced by whether the combination of downstaging therapy is implemented.

Another important issue is the endpoint of downstaging and criteria for post-downstaging liver resection, which must be defined more precisely. It is generally believed that a reduction in tumor size may be an effective evaluation indication (20, 21, 34). A few researchers have adopted the criteria of partial remission (PR) for resection after downstaging (21, 27). However, whether the subsequent HR needs to be removed when the tumor disappears on imaging remains controversial. Shi et al. (2012) considered that surgical resection should be performed under these conditions because clinical complete response (CR) does not represent pathological CR (27). Residual viable cancer cells may lead to a high recurrence rate (10). According to Hamaoka et al. (2017) (34) and Lee et al. (2019) (36), successful conversion to surgery is considered to be a factor of favorable prognosis. In contrast, Zhang et al. (2016) reported that salvage surgery after TACE for unresectable HCC has an OS benefit only in patients with a PR to TACE, while those achieving CR group do not show improvement (32). This might be due to the role of downstaging in the selection of biological aggression. The presence of vascular invasion, multiple tumors, and high alpha-fetoprotein levels are regarded as risk

factors of survival in patients with resectable HCC as well as downstaging (2, 21, 31, 35). Therefore, downstaging may serve as a screening tool to identify patients who might benefit from surgery. Since conventional criteria for HR are based on HCC morphology, downstaging, which can predict HCC biology, will be more favorable.

The limitation of high heterogeneity among different downstaging strategies for HCC should also be considered. The selection of a treatment strategy is based merely on the habit at a single center. Additionally, no RCT has been performed; therefore, the grade of evidence was weak.

To date, there has been surrounding the best strategy for unresectable HCC. The evidence of HR mainly came from small published series, having demonstrated useful attempts. Based on this meta-analysis, operable patients with unresectable HCC may be screened for downstaging. Surgical resection after successful downstaging can maximize the improvements in the prognosis of patients with unresectable HCC, bringing hope for patients initially considered incurable. With the rapid advancements in LRT in recent years, the emergence of novel targeted therapies, especially immunotherapy, has tremendously facilitated non-surgical treatments for HCC, suggesting a potential role for downstaging. In the future, prospective trials with large sample sizes on these new methods are expected to provide reasonable guidance and inspire more effective strategies for downstaging approaches. Future criteria should include a clear downstaging endpoint and molecular biological information and markers.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

Ethical review and approval was not required for the study on human participants in accordance with the local legislation and institutional requirements. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

Conceptualization: LeL and JY. Study selection and data extraction: XC and LiL. Statistical analysis: XC. Writing—original draft: XC and LiL. Writing—review and editing: LeL and JY. Final approval of the manuscript: all authors. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2021.740762/full#supplementary-material

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Lenvatinib Plus PD-1 Inhibitors as First-Line Treatment in Patients With Unresectable Biliary Tract Cancer: A Single-Arm, Open-Label, Phase II Study

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Objective: We investigated lenvatinib plus programmed cell death-1 (PD-1) inhibitors as a first-line treatment for initially unresectable biliary tract cancer (BTC).

Methods: In this Phase II study, adults with initially unresectable BTC received lenvatinib (body weight ≥60 kg, 12 mg; <60 kg, 8 mg) daily and PD-1 inhibitors (pembrolizumab/tislelizumab/sintilimab/camrelizumab 200 mg or toripalimab 240 mg) every 3 weeks. Primary endpoints were objective response rate (ORR) and safety. Secondary endpoints included surgical conversion rate, disease control rate (DCR), event-free survival (EFS), overall survival (OS) and tumor biomarkers.

Results: Among 38 enrolled patients, the ORR was 42.1% and the DCR was 76.3%. Thirteen (34.2%) patients achieved downstaging and underwent surgery, six of whom (46.2%) achieved a major pathologic response (n=2) or partial pathologic response (n=4) in the primary tumor. In total, 84.2% of patients experienced ≥1 treatment-related adverse event (TRAE), 34.2% experienced a Grade ≥3 TRAE and no treatment-related deaths occurred. After a median follow-up of 13.7 months the median EFS was 8.0 months (95% CI: 4.6–11.4) and the median OS was 17.7 months (95% CI: not estimable).

Conclusions: Lenvatinib plus PD-1 inhibitors showed promising anti-tumor efficacy in patients with initially unresectable BTC and was generally well tolerated.
Clinical Trial Registration: www.chictr.org.cn, ChiCTR2100044476.

Keywords: biliary tract cancer, lenvatinib, PD-1 inhibitors, first-line treatment, conversion surgery

INTRODUCTION

Biliary tract carcinomas (BTC) are a group of cancers that include intrahepatic cholangiocarcinoma (ICC), extrahepatic cholangiocarcinoma (ECC) and gallbladder cancer (GBC), and account for 10-15% of primary liver malignancies (1, 2). As a highly aggressive malignant tumor originating from the bile duct epithelium, BTCs are associated with a particularly low life expectancy of around one year. Although radical surgical resection is a potentially curative therapy for BTC, over half of patients have unresectable disease at diagnosis (3). If patients with unresectable BTC are able to achieve adequate downstaging through effective systemic therapy, they may have an opportunity to undergo surgical resection (a 'conversion resection') and therefore achieve long-term survival. The conversion therapy treatment strategy is successfully utilized in non-liver cancers and has shown promising results in hepatocellular carcinoma (4). However, despite recent advances in the multidisciplinary treatment of BTC, there remains a lack of effective treatment strategies for achieving secondary resection for patients with this disease.

Gemcitabine combined with cisplatin is currently recommended as the standard first-line therapy for patients with advanced BTC (5). However, the survival outcomes associated with this treatment are suboptimal, with a median overall survival (OS) of approximately 6-8 months (6). In second-line therapy or later, no targeted therapy or immune therapy has yet been approved for advanced BTC. Inhibitors of programmed death-1 (PD-1) and programmed death ligand-1 (PD-L1) have shown promising antitumor efficacy across multiple cancer types (7–9). However, randomized trials of anti-PD-1 and anti-PD-L1 monoclonal antibodies in unresectable or recurrent BTC have so far failed to demonstrate a higher treatment response or survival benefit compared with standard chemotherapies (10). For example, in the KEYNOTE-158 study, patients with advanced BTC receiving pembrolizumab monotherapy achieved an objective response rate (ORR) of 5.8% while the median OS was 7.4 months, and many patients did not achieve any clinical benefit (11, 12). These findings suggest that tumor resistance to anti-PD-1 antibodies limits the proportion of patients with BTC who can benefit from this therapy.

Lenvatinib is a tyrosine kinase (TKI) inhibitor of vascular endothelial growth factor receptor (VEGFR) 1-3, fibroblast growth factor receptor (FGFR) 1-4, platelet derived growth factor receptor a (PDGFRa), RET, and KIT (13). Preclinical studies have demonstrated that lenvatinib can enhance the anti-tumor activity of T lymphocytes in the tumor microenvironment through anti-angiogenic effects, thereby enhancing the anti-tumor effect of anti-PD-1/PD-L1 antibodies (14, 15). Results from mouse models further showed that TKIs combined with PD-1 inhibitors result in greater tumor regression and a higher response rate compared with either treatment alone (16). Clinically, lenvatinib in combination with PD-1 inhibitors has been regarded as a breakthrough therapy in unresectable melanoma (17), hepatocellular carcinoma (18) and renal cell carcinoma (19). Recently, a prospective study (NCT03895970) reported that treatment with lenvatinib plus pembrolizumab (LEP) in patients with refractory BTC resulted in promising antitumor activity (20). However, there are currently no published data on the first-line using combined treatment with TKI inhibitors and PD-1 inhibitors in BTC.

This prospective Phase II trial was conducted to evaluate the efficacy and safety of first-line lenvatinib plus PD-1 inhibitors in patients with initially unresectable BTC and to explore the feasibility of conversion surgery following this therapy.

MATERIALS AND METHODS

Study Design and Patients

This was an open-label, single-center, phase II trial (Chictr.org identifier: ChiCTR2100044476) that included adult (≥18 years) patients with a histologically confirmed diagnosis of biliary tract adenocarcinoma (including ICC, ECC or GBC) that was initially considered unresectable. Initially unresectable BTC was defined as patients for whom R0 resection could not be achieved, even through aggressive surgical procedures, and was determined by a multi-disciplinary team based on imaging evaluation of hepatic artery and portal vein invasion, tumor size, tumor location, remnant liver volume and presence of extrahepatic metastasis. Eligible patients were also required to have ≥1 measurable target lesion according to Response Evaluation Criteria in Solid Tumors (RECIST) v1.1, Child Pugh class A liver function and an Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1. Patients were excluded if they had received prior chemotherapy, TKI therapy, anti-PD-1 or anti-PD-L1 agents, and had a diagnosis of immunodeficiency or active autoimmune disease or a history of bleeding disorders.

The study protocol was approved by the ethics committee of the Second Affiliated Hospital of Zhejiang University of Medicine and the study was conducted in accordance with the Declaration of Helsinki and principles of Good Clinical Practice. All patients provided written informed consent before inclusion.

Systemic Therapy

Eligible patients received lenvatinib (body weight ≥60 kg, 12 mg; <60 kg, 8 mg) orally once daily as well as a PD-1 inhibitor intravenously every 3 weeks. Five different PD-1 inhibitors were utilized based on patient preference (pembrolizumab 200 mg, tislelizumab 200 mg, sintilimab 200 mg, camrelizumab 200 mg or toripalimab 240 mg). Treatment was continued until unacceptable toxicity, radiologically confirmed disease progression assessed by RECIST v1.1 or withdrawal of consent.

Surgical Procedures

For patients who achieved sufficient downstaging during systemic therapy to become eligible for surgery, resection was performed 1 to 3 weeks after the last cycle of treatment. Patients included for conversion resection were evaluated as partial response (PR) or stable disease (SD) for at least 2 months. If patients achieved tumor regression (regressed SD or PR) or lymph node regression, and R0 resection could be achieved with sufficient remnant liver volume but have Grade ≥3

TRAEs, we considered resection for them as soon as possible. However, patients with persistent tumor shrinkage without severe TRAEs will continue to accept the conversion therapy. The criteria for successful surgical conversion included: (1) significant tumor regression (at least partial response or regressed stable disease); (2) Child-Pugh class A liver function; (3) no distant metastasis; (4) R0 resection was possible; (5) ECOG status of 0 or 1; (6) sufficient future liver remnant if hepatic resection was required. Radical resection including systematic lymphadenectomy, partial hepatectomy, combined vascular resection and revascularization were performed according to the extent of tumor invasion during the operation.

Measurements and Endpoints

Data collected at baseline included patient sex, age, pathological type, clinical TNM staging and carbohydrate antigen 19-9 (CA199) level. The normal value of CA199 was based on our institutional standard. Contrast-enhanced CT or MRI was used to assess the tumor at baseline and every 8 weeks (± 2 weeks) thereafter using RECIST v1.1. The primary endpoints were ORR and safety. Secondary outcomes included conversion rate, disease control rate (DCR), event-free survival (EFS), OS and postoperative complications. EFS was defined as the time from initiation of systemic therapy to the occurrence of progressive disease or death from any cause. Safety was assessed throughout the entire study and for 30 days after treatment discontinuation, and during the postoperative period. Treatment-related adverse events (TRAEs) were graded based on the National Cancer Institute Common Terminology Criteria for Adverse Events, version 4.0.

Pathological Assessments

Surgical tissue specimens were staged according to the American Joint Committee on Cancer (AJCC Cancer Staging Manual. 8th) (21). Hematoxylin and eosin (HE) staining was performed to evaluate the percentage of residual viable tumor in the primary tumor, and ≤10% viable tumor in the treated tumor bed was considered to be a major pathological response (MPR). A partial pathologic response (pPR) was defined as >10% and ≤50% residual viable tumor by chemotherapy criteria while a pathologic nonresponse was defined as >50% residual viable tumor (22).

Biomarker Analysis

Fine needle aspiration specimens were obtained from each patient before initiation of systemic treatment. Immunohistochemistry was performed to detect the expression of PD-L1 using Dako 22C3 (Dako Monoclonal Mouse Anti-Human PD-L1, Clone 22C3) on tumor biopsy samples. PD-L1 expression was evaluated using isolated tumor cells and certified by a senior pathologist in our hospital. Whole exon sequencing (WES) was conducted using the SureSelect Human All Exon V6 kit (Agilent, Santa Clara, CA, USA). Genomic alterations, including microsatellite stability status, single base substitutions, short and long insertions/deletions (INDELS), copy number variants, and gene rearrangement and fusions, were assessed. Tumor mutation burden (TMB) was determined by analyzing somatic mutations including coding base substitutions and INDELs according to the megabase (Mb).

Statistical Analysis

Assessment of TRAEs, postoperative complications and feasibility analyses were conducted in all patients who received at least one dose of lenvatinib plus PD-1 inhibitors. Continuous variables were expressed as median (range) and between-group differences were compared using a Student's t-test or Mann-Whitney U test. Categorical variables were presented as number of patients and associated percentage. The ORR, DCR and duration of response (DoR) and corresponding 95% CIs were calculated using the Clopper-Pearson method. Chi-squared or Fisher exact tests was used to evaluate associations between biomarkers and treatment response. EFS and OS were estimated using the Kaplan-Meier method. All statistical analyses were performed using R software (version 3.6.2) or GraphPad Prism software (version 7).

RESULTS

Patients

Between March 1, 2018 and May 31, 2021, a total of 38 patients were enrolled in the study. Patient demographics and baseline characteristics are summarized in **Table 1**. At the cut-off date for this analysis (May 31, 2021), a total of 23 (60.5%) patients had discontinued treatment and 15 (41.7%) remained on treatment (**Figure S1**). The most common reason for discontinuing both study treatments was confirmed progressive disease or death (n=18). Three patients received second-line combined chemotherapy and two refused to continue treatment due to economic reasons. A total of six patients were still receiving combination treatment with lenvatinib and anti-PD-1 antibodies after surgery despite confirmed tumor relapse. The patient time on treatment is summarized in **Figure 1B**.

Safety

Dose reductions and treatment discontinuations due to TRAEs were experienced by five (13.9%) and one (2.8%) patients, respectively. Four patients had a lenvatinib dose reduction from 8 mg to 4 mg per day due to lenvatinib-related toxicities. One patient discontinued lenvatinib plus PD-1 inhibitor because of treatment-related cerebral hemorrhage.

During the study, 84.2% of patients experienced ≥1 TRAE, and there were no treatment-related deaths (**Table S1**). Most TRAEs were of a low grade and were easily managed. The most common TRAEs of any Grade were fatigue (n=14), anorexia (n=8), alanine aminotransferase (ALT) elevation (n=7) or aspartate aminotransferase (AST) elevation (n=7), rash (n=6), hypertension (n=5) and hoarseness (n=5). Grade ≥3 TRAEs occurred in 34.2% of patients and the most common were fatigue (n=5) and hypertension (n=3). One patient experienced Grade 4 cerebral hemorrhage caused by hypertension, which was the most serious TRAE observed, although the patient was successfully treated for this adverse event.

TABLE 1 | Summary of patient demographics and baseline characteristics.

Characteristic, n (%)	N = 38
Age, years, median (range)	62.50 (57.27-64.52)
Sex	
Male	14 (36.8)
Female	24 (63.2)
PD-1 antibody received	
Pembrolizumab	3 (7.9)
Toripalimab	12 (31.6)
Tislelizumab	11 (28.9)
Sintilimab	11 (28.9)
Camrelizumab	1 (2.6)
ECOG CPS	
0	26 (68.4)
1	12 (31.6)
Tumor subtype	
Intrahepatic cholangiocarcinoma	20 (52.6)
Extrahepatic cholangiocarcinoma	5 (13.2)
Gallbladder cancer	13 (34.2)
TNM stage[a]	
II	5 (13.2)
III	19 (50.0)
IV	14 (36.8)
CA199 level, U/mL	
< 111	18 (50)
≥ 111	18 (50)
Previous therapy	
Radical surgical resection	3 (8.3)
ERCP or PTCD	2 (5.6)

[a]*Clinical staging was based on the 8th edition of the American Joint Committee on Cancer (AJCC) Staging Manual.*

CA199, carbohydrate antigen 199; CPS, combined positive score; ECOG, Easter Co-operative Oncology Group; ERCP, endoscopic retrograde cholangiopancreatography; PTCD, percutaneous transhepatic cholangiography and drainage.

Radiographic Response Evaluation

All patients had at least one radiological evaluation. A total of 16 (42.1%, 95% CI: 25.7% to 58.6%) patients achieved a partial response, 13 (34.2%, 95% CI: 18.4% to 50.0%) achieved stable disease and no patients achieved a complete response (**Table 2** and **Figure 1A**). The ORR was 42.1% (95% CI: 25.7% to 58.6%). Among the 16 patients who achieved a partial response, nine (56.3%) were confirmed as an objective response. The DCR was 76.3 (95% CI: 62.2% to 90.5%).

Surgery

Of 38 evaluable patients, 34.2% (n=13) achieved adequate tumor volume reduction were considered eligible for resection (**Figure S2**). The median conversion time from initiation of systemic therapy to surgery was 5.5 months (95% CI: 3.8 to 7.1). Among patients who underwent conversion surgery, 12 (92.3%) achieved a R0 resection, and one underwent palliative resection due to abdominal tumor distant metastasis (**Figure 2**). Representative images from two patients who successfully underwent conversion surgery and achieved good postoperative outcomes are presented in **Figure 3**.

Of the patients who underwent resection, six experienced postoperative complications, including two cases of biliary leakage, two cases of pleural effusion, one case of delayed liver function recovery and one case of upper gastrointestinal bleeding. All patients undergoing surgery were successfully discharged after postoperative care.

Follow-up

After a median follow-up of 13.7 (95% CI: 9.7 to 17.8) months, the 1-year OS rate was 47.4% (18/38), and 65.8% of patients were still alive. The median EFS was 8.0 months (95% CI: 4.6 to 11.4) and the median OS was 17.7 months (95% CI: not estimable) (**Figure 4**). Among the 13 patients who underwent conversion surgery, the median EFS was 13.5 months (95% CI: 13.0 to 14.0).The median recurrence-free survival (RFS) and median OS were not reached (**Table S2**). Among patients who only received systemic therapy, the median EFS was 4.6 months (95% CI: 0.8 to 8.4) and the median OS was 12.4 months (95% CI: 8.5 to 16.3). Compared to patients receiving only systemic therapy, patients who successfully achieved conversion resection had a longer EFS and OS (**Table 2**). One patient who underwent conversion surgery had survived for 39.0 months as of the cut-off. The change in tumor size for patients in the conversion surgery group and no-surgery group is shown in **Figure 1C**.

Pathologic Findings

Among the 13 patients who underwent a conversion resection, two (15.4%) achieved a MPR in the primary tumor, and four (30.8%) achieved a pPR (**Figure 2**). No patient achieved a complete pathological response (no viable tumor cells). The median degree of pathological regression in the primary tumor were -30% (range: -26.8% to -65.6%). No post-surgical relapse was observed in patients who achieved a MPR or pPR. However, five (71.4%) patients who were pathological non-responders experienced disease recurrence. Pathological analysis of resected tumor specimens revealed varying degrees of post-treatment necrosis and treatment-related immune activation. In primary tumors categorized as MPR or pPR, we observed a large number of tumor-infiltrating lymphocytes and macrophages, which were especially notable in the surrounding adjacent tissue. However, these tumor immune response-related cells were rarely observed in patients who were pathologic non-responders. Necrosis was found mainly in the middle area of the tumor and finally replaced by fibrosis (**Figures 3B, E**).

PD-L1 Expression Analysis

PD-L1 expression was evaluated in pretreatment biopsy samples obtained from 29 patients. Immunohistochemistry showed that objective responses were achieved by 10 (61.1%) of 18 patients with positive PD-L1 (combined positive score [CPS] ≥1%) and five (45.5%) of 11 patients with negative PD-L1 (CPS <1%) (**Figure S3A**). Consequently, patients with positive PD-L1 expression showed significantly prolonged survival outcomes for both event free survival (EFS) (P=0.009) and OS (P=0.013), suggesting that PD-L1 expression is a potential prognostic factor (**Figures 5C, E**). Moreover, in the subgroup of patients who underwent resection, four PD-L1 positive samples were identified in the five patients (80.0%) who achieved a MPR or pPR, while only 57.1% of patients with pathologic non-response were positive for PD-L1. In an immune response analysis, patients with positive PD-L1 expression were shown to have more tumor infiltrating lymphocytes clustered around the tumor (**Figure 3B**).

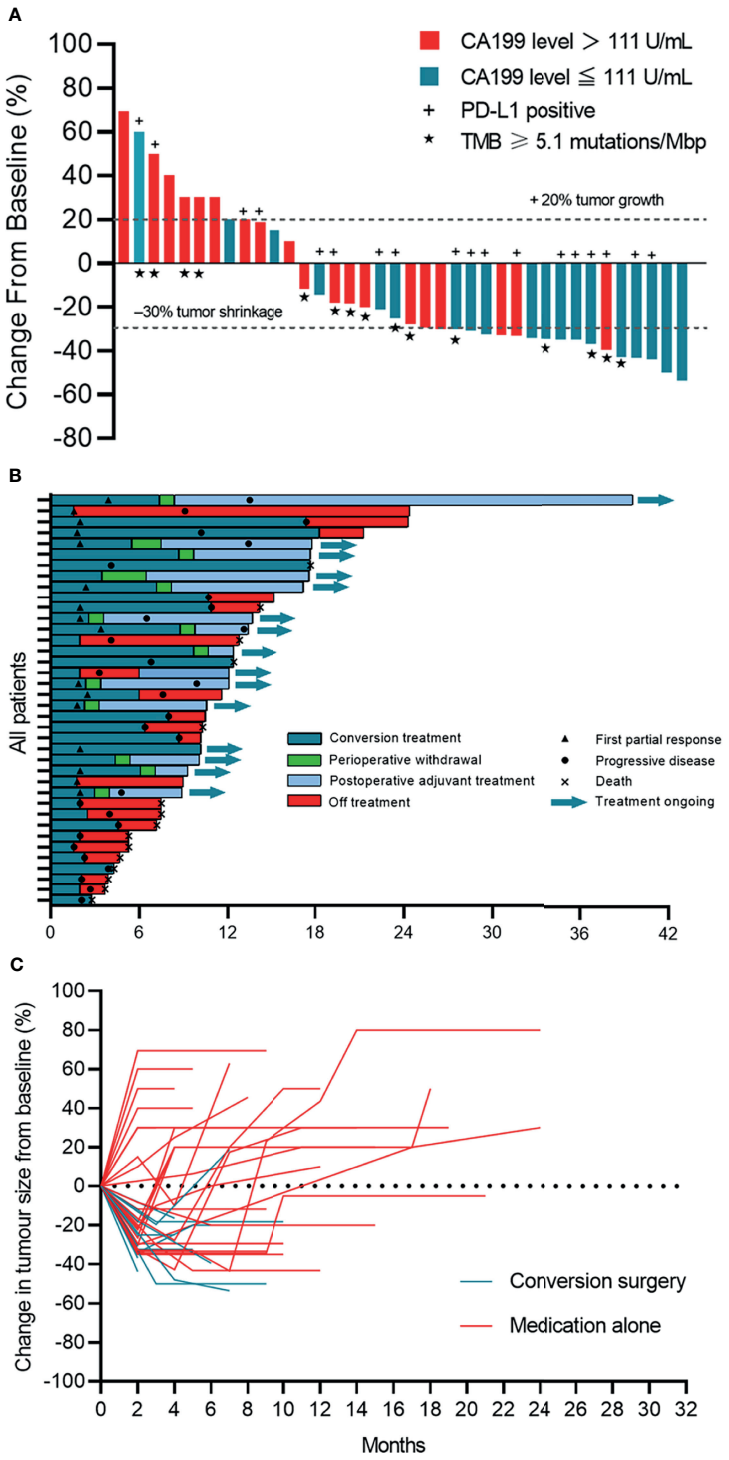

FIGURE 1 | Tumor response. **(A)** Waterfall plot of maximum percent change in tumor size from baseline in each patient as measured by RECIST (version 1.1). **(B)** Time on treatment. **(C)** Longitudinal change in tumor size from baseline. Patients who underwent surgery stopped follow-up after the primary tumor was removed.

Genomic Analysis

We performed WES on pre-treatment tumor samples obtained from 29 patients who had adequate available tissue. The relationship between clinical response to lenvatinib plus anti-PD-1 therapy and underlying molecular profiles was investigated. A total of 3124 mutations were detected. Statistical analysis showed that mutations in DNAH17, SSPO or ARID1A were significantly associated with low ORR

TABLE 2 | Summary of tumor response and survival outcomes.

Therapeutic response assessment	N = 38
ORR[a], % (95% CI)	42.1 (25.7 to 58.6)
Confirmed ORR[a,b], % (95% CI)	9 (23.7, 9.5 to 37.8)
Best overall response[a,b], n (%) [95% CI]	
CR	0
PR	16 (42.1) [25.7 to 58.6]
SD	13 (34.2) [18.4 to 50.0]
PD	9 (23.7) [9.5 to 37.8]
Conversion rate, n (%) [95% CI]	13 (34.2) [18.4 to 50.0]
Conversion time, months, median (95% CI)	5.5 (3.8 to 7.1)
DCR[b], % (95% CI)	76.3 (62.2 to 90.5)
EFS[c], months, median (95% CI)	8.0 (4.6 to 11.4)
6-month EFS rate, % (95% CI)	63.2 (47.1 to 79.2)
1-year EFS rate, % (95% CI)	21.1 (7.5 to 34.6)
EFS for patients who underwent surgery, months, median (95% CI)	13.5 (13.0 to 14.0)
EFS for patients who did not undergo surgery, months, median (95% CI)	4.6 (0.8 to 8.4)
OS[c], months, median (95% CI)	17.7 (NR)
6-months OS rate, % (95% CI)	81.6 (68.7 to 94.5)
1-year OS rate, % (95% CI)	47.4 (30.7 to 64.0)
OS for patients who underwent surgery, months, median (95% CI)	NR
OS for patients who did not undergo surgery, months, median (95% CI)	12.4 (8.5 to 16.3)

[a]*Treatment response was evaluated according to RECIST v1.1.*
[b]*Calculated using exact method of binomial distribution (Clopper-Pearson method).*
[c]*Kaplan-Meier method was used for estimating EFS and OS.*
CI, confidence interval; CR, complete response; DCR, disease control rate; EFS, event-free survival; ORR, objective response rate; PD, progressive disease; PR, partial response; NR, not reached; OS, overall survival; SD, stable disease.

(DNAH17, 6.9% vs 44.83%, *P*=0.02; SSPO, 6.90% vs 44.83%, *P*=0.02; ARID1A, 3.45% vs 48.28%, *P*=0.04) (**Figure 6B**). Prognosis analysis revealed that patients with PI3K-pathway mutations had shorter EFS (median EFS, 6.5 months vs 10.9 months, *P*=0.074) (**Figure S4C**) and OS (median OS, 12.4 months vs not reached, *P*=0.037) (**Figure 5G**) compared with the PI3K-pathway wild-type group. Genetic alterations and frequencies identified by WES are summarized in **Figure 6A** and **Table S3**.

TMB

TMB was determined by analyzing somatic mutations within the coding region of the human genome though WES. The median TMB was 5.10 muts/Mb in 29 patients with available data. Ten (71.4%) of 14 patients with a low TMB (median 5.10 as the cutoff value) and five (33.3%) of 15 patients with a high TMB achieved objective responses (**Figure 5B**). In addition, patients with a low TMB had a significantly longer OS than those with a high TMB (*P*=0.003) (**Figure 5F**).

Other Biomarker Analyses

Chi-squared showed that pretreatment serum CA199 level was associated with treatment response. Using 111 U/mL as the cutoff value, patients with lower CA199 levels had a significantly higher ORR (68.4%) (**Figure 5A**) and prolonged OS compared to the high CA199 level group (*P*=0.028) (**Figure 5D**). In the low CA199 level group, 10 of 18 patients (55.6%) underwent resection, and no deaths had occurred in these patients at the data cut-off.

Interestingly, compared with patients with ECC or ICC, those with GBC had a higher ORR (61.5%) and a higher surgical conversion rate (46.2%). However, given the small sample size, further study is warranted in a larger cohort of GBC.

DISCUSSION

Locally advanced and metastatic BTCs are generally considered unresectable and lack effective treatment options. Despite the progress made in other cancers, no TKI or PD-1 inhibitor has been approved for the treatment of advanced BTC to date. Preclinical evidence suggests that combined treatment with a TKI inhibitor and anti-PD-1 or anti-PD-L1 antibodies induces additive antitumor effects. Furthermore, a clinical study in refractory BTC also reported higher ORR and longer OS with TKI or PD-1 inhibitor combination therapy compared with prior findings for TKI or PD-1 inhibitor monotherapy (20). The present study provides clinical evidence that combination therapy with a TKI plus PD-1 inhibitor in the first-line treatment of advanced BTC may provide a robust anti-tumor effect and allow a proportion of patients to achieve downstaging and conversion to surgical treatment.

To our knowledge, this is the first trial of lenvatinib plus PD-1 inhibitors for the first-line treatment of BTC. We found that this combination therapy is relatively well tolerated, with TRAEs experienced by 84.2% of patients and no treatment-related deaths. In addition, a total of 34.2% of patients experienced Grade ≥3 TRAEs, which was lower than reported in a previous study of LEP in 32 Chinese patients with refractory BTC in which 100% of patients experienced TRAEs and 59.3% experienced Grade ≥3 AEs (20). The most common TRAEs reported with LEP in this previous study were fatigue,

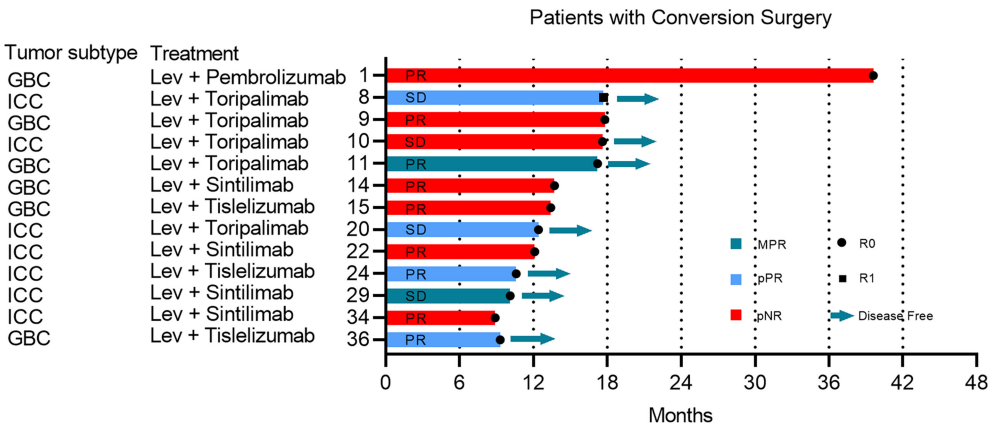

FIGURE 2 | The complete course of conversion treatment and post-surgery outcome. No more than 10% of viable tumor in the treated tumor beds were considered to have had a major pathological response (MPR). Partial pathologic response (pPR) was defined as more than 10% and less than 50% residual viable tumor by chemotherapy criteria while pathologic nonresponse (pNR) was defined as more than 50% residual viable tumor. The preoperative radiographic response of each patient was marked in the bar. ICC, intrahepatic cholangiocarcinoma; GBC, gallbladder cancer; PD, progressive disease; PR, partial response; SD, stable disease; Lev, lenvatinib.

hypertension and anorexia, which were similar with those observed in the present study. Furthermore, of the 13 patients in the present study who underwent surgery, only six experienced postoperative complications, while all patients who underwent surgery were successfully discharged after postoperative care. Generally, the combined regimen of lenvatinib plus PD-1 inhibitors was well tolerated and all toxicities were manageable.

In terms of efficacy, we found a DCR of 76.3% and an ORR of 42.1%, with a median EFS of 8.0 months, a median OS of 17.7 months and a 1-year OS rate of 47.4%. These findings suggest that combination therapy exerts a better anti-tumor effect than TKI or PD-1 inhibitor monotherapy in patients with advanced BTC. Furthermore, the surgical conversion rate of this cohort reached 34.2% and the successful R0 resection rate was 31.6%. There have been no prior reports of conversion therapy with TKIs or PD-1 inhibitors in BTC; however, previous studies demonstrated that chemotherapy can lead to downstaging and conversion to surgery. A recent systematic review including patients from 10 trials indicated a surgical conversion rate of 17.3% (27/132), and that 23 of the 27 patients who underwent surgery were alive at the last reported follow-up of this study (23). Notably, the conversion rate reported for chemotherapy in this prior systematic review was lower than observed in our study (17.3% vs 31.6%). A further study conducted in South Korea assessed chemoradiotherapy (CRT) for downstaging unresectable ICC to resectable lesions and reported an ORR of 25% and conversion rate of 12.5% (24). Eight patients in this study were able to receive a curative resection after CRT and showed significantly improved OS compared to patients treated with CRT alone (3-year OS: 50% vs. 11.2%, respectively, P=0.012). Although patients in this prior study who were able to undergo surgery achieved prolonged survival, the conversion rate was relatively low compared with the present study in which the R0 resection rate was 31.6%. Therefore, the combination of lenvatinib plus PD-1 inhibitors outperformed both chemotherapy and CRT in terms of conversion to surgical resection for patients with advanced BTC.

Basic laboratory research has revealed that the immunologic effects of the PD-1 pathway on T cell priming, effector function and exhaustion suggest distinct mechanisms underlying surgical conversion with immunotherapy versus chemotherapy. Chemotherapy achieves downstaging and conversion by reducing tumor burden preoperatively, whereas immunotherapy can enhance systemic immunity against tumor antigens, thereby also inhibiting postoperative recurrence by eliminating micrometastatic tumors (25). Moreover, the inhibition of tyrosine kinases can enhance the function of T lymphocytes in the tumor microenvironment through anti-angiogenic effects, thereby enhancing the anti-tumor effect of anti-PD-1/PD-L1 antibodies (26). Therefore, combining TKIs with PD-1 inhibitors has been shown to promote the anti-tumor effect of T cells in the immune system, whereas chemotherapy inhibits this mechanism by depleting regulatory T cells. This may explain why the clinical efficacy of lenvatinib plus PD-1 inhibitors in this study exceeded that reported in studies of conversion therapy in BTC using chemotherapy.

We evaluated the pathological response of 13 surgically resected tumor specimens. Although the MPR rate was low (15.4%) on histological examination, there was an association with prognosis. Among patients with primary tumor shrinkage of more than 50% (MPR and pPR), no postsurgical relapse was observed by the cut-off date for this analysis, while 71.4% (5/7) of those with a pathologic non-response experienced tumor recurrence. One patient who experienced stable disease assessed by RECIST 1.1 achieved 95% tumor shrinkage in postoperative pathological analysis. A correlation between pathologic response and improved recurrence-free survival or

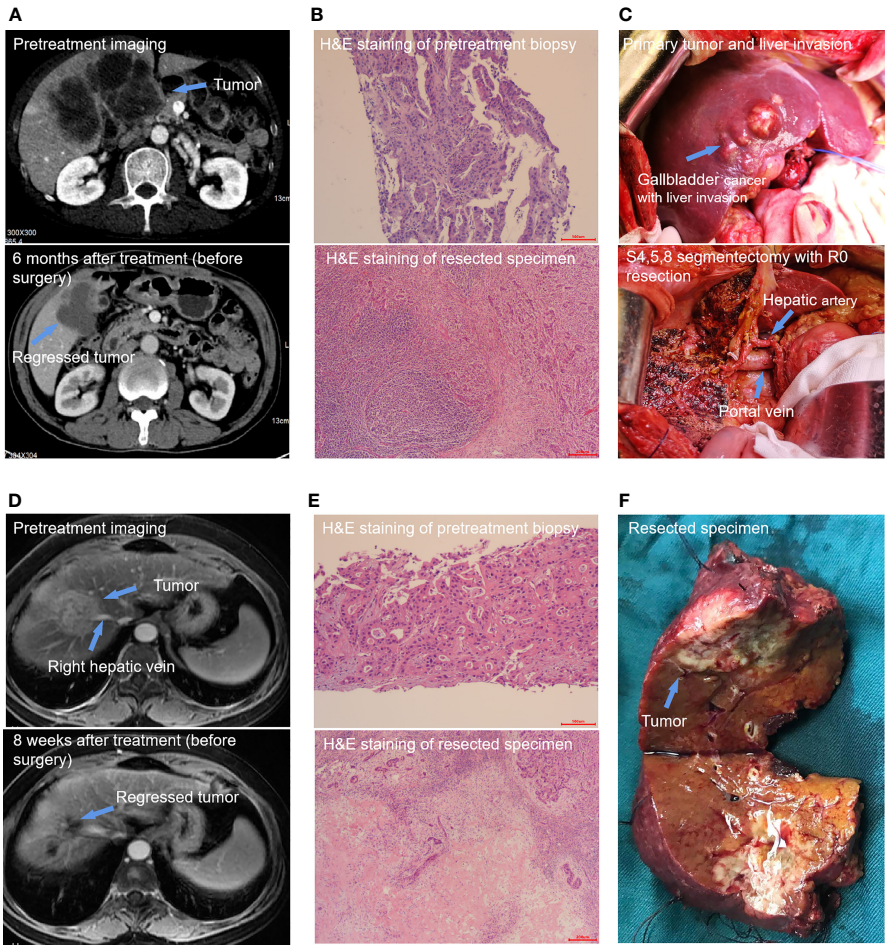

FIGURE 3 | Two special cases report. (1) Patient 11 was a 65-year-old male patient with stage IIIB gallbladder cancer. Pretreatment contrast-enhanced computed tomography (CT) imaging of the abdomen showed a huge tumor including the primary tumor and liver invasion. The tumor was significantly shrunk after 6 months of conversion treatment **(A)**. Hematoxylin and eosin staining of resected specimen showed a MPR and a plenty of lymphocyte infiltration **(B)**. S4,5,8 segmentectomy with R0 resection **(C)**. (2) Patient 24 was a 65-year-old female patient with stage II intrahepatic cholangiocarcinoma. The pretreatment Magnetic Resonance Imaging (MRI) imaging showed a primary tumor mass of 5.2 cm in diameter and has invaded the main branch of the right hepatic vein. A scan performed before surgery showed that most of the primary tumors had appeared necrosis and shrunk significantly **(D)**. The pathologic images shown are representative sections of the patient before conversion therapy and large amount of post-treatment necrosis and tumor-infiltrating lymphocytes and macrophages were found in the primary tumor postoperation **(E)**. Specimen with R0 resection **(F)**.

OS has been shown in neoadjuvant studies for several cancer types (27–29). These findings prove that postoperative pathological analysis has certain advantages in predicting postoperative tumor recurrence, and this has been a longstanding surrogate endpoint in studies of advanced BTC.

The identification of biomarkers to evaluate tumor response in the conversion setting represents an important secondary aim of this study. In this regard, we found that histopathological type of BTC was associated with treatment response and prognosis. Patients with GBC (n=13) had an ORR of 61.5% which was higher than patients with ICC (40%) or ECC (0%). Six patients (46.2%) with GBC underwent resection, while none with ECC achieved an objective response or converted to surgical resection. Although this result is interesting, considering the small sample size and possible selection bias, further study in a larger cohort of BTC is needed.

CA199 is a commonly used biomarker for predicting recurrence of BTC after neoadjuvant chemotherapy. Lehrke et al. retrospectively analyzed data from 132 patients with perihilar cholangiocarcinoma who underwent liver transplantation after neoadjuvant chemoradiotherapy. They found that the postoperative recurrence and mortality rates of patients with CA199 level ≥200 U/L were 2.3 times and 2.4 times that of patients with CA199 level <200 U/L, respectively (30). Similarly, in the present study, we found that pretreatment CA199 level was closely related to response to therapy. Patients with low CA199 levels had a higher ORR (68.4%) and OS than the high CA199 group. These findings suggest that CA199 level

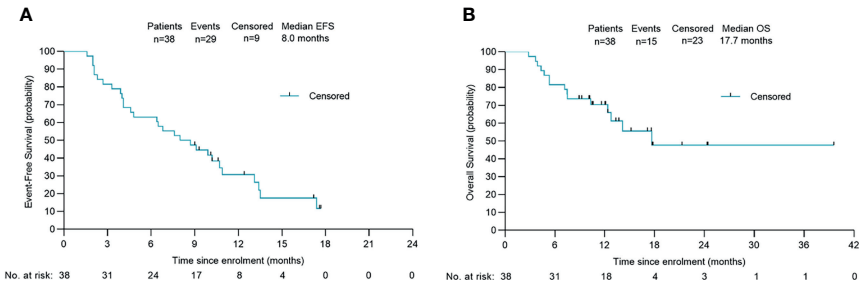

FIGURE 4 | Survival outcomes of 38 patients. Kaplan-Meier plots of overall event-free survival **(A)** and overall survival **(B)**. Probability of survival is shown at indicated time points. Numbers of patients at risk at indicated time points are shown below the x-axis. Censored patients are marked with a vertical line in the graph. EFS, event-free survival; OS, overall survival.

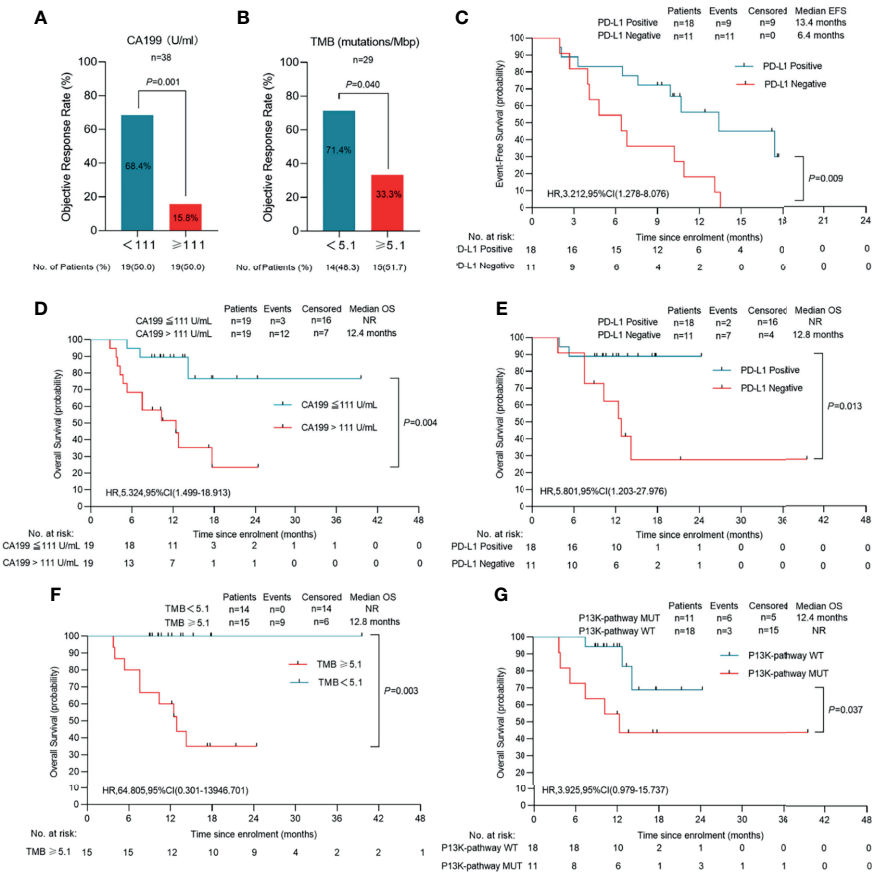

FIGURE 5 | Clinical response in relation to tumor biomarkers in patients with initial unresectable BTC. **(A)** The cutoff value of CA199(CA199 = 111 U/mL) is three times the normal value of our institution (n=38). **(B)** TMB was calculated by summing up somatic mutations within the coding regions by whole-exon sequencing. A TMB of 5.1 mutations per million base pairs (Mbp) was the cutoff value (n=29). **(C)** Event-free survival of patients of PD-L1 positive or PD-L1 negative (n=29). **(D)** Overall survival of patients of CA199 ≤ 111 U/ml and CA199 > 111U/ml (n=38). **(E)** Overall survival of patients of PD-L1 positive or PD-L1 negative (n=29). **(F)** Overall survival of patients of TMB<5.1 mutations/Mbp or TMB ≥ 5.1 mutations/Mbp (n=29). **(G)** Overall survival of patients of P13K-pathway wide type and P13K-pathway mutation (n=29). Probability of survival is shown at indicated time points. Censored patients are marked with a vertical line in the graph. Numbers of patients at risk at indicated time points are shown below the x-axis. NR, not reached.

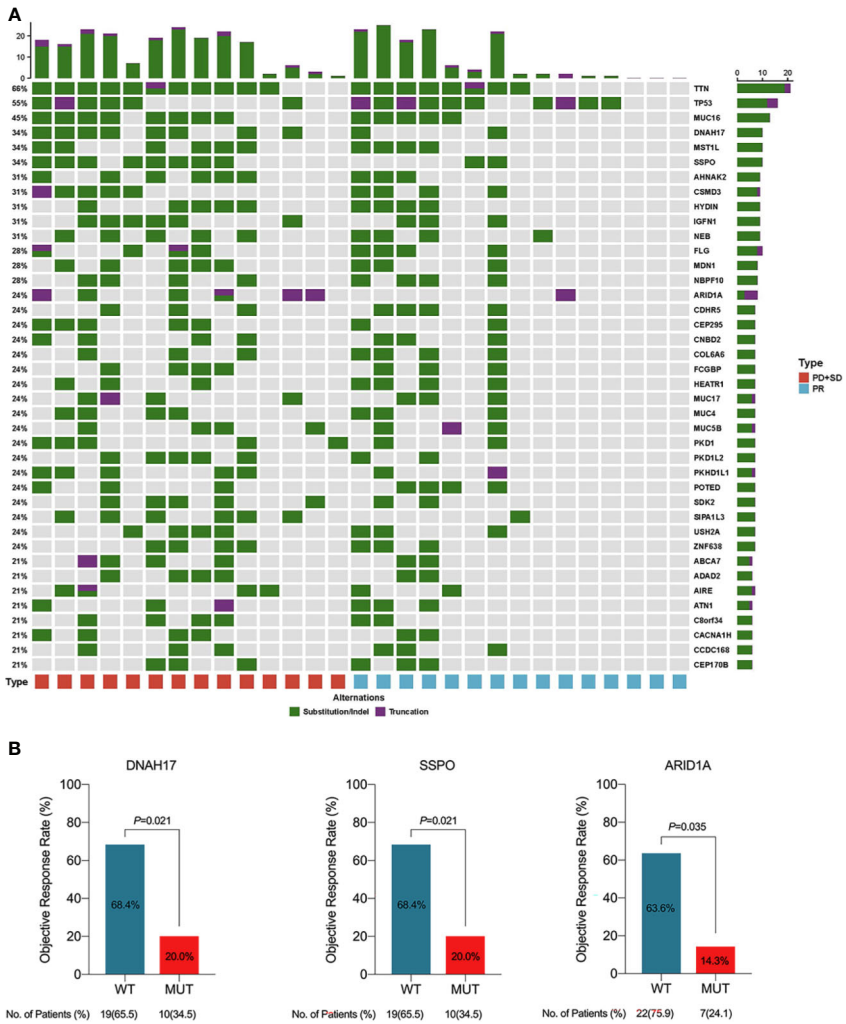

FIGURE 6 | **(A)** Genetic alternations and frequencies identified by whole exome sequencing (WES) from 29 available patients. **(B)** Clinical response in relation to gene mutations in patients with initial unresectable BTC. PD, progressive disease; PR, partial response; SD, stable disease; WT, wide type; MUT, mutation.

could be used to screen patients with BTC and identify those who are likely to respond to combination therapy.

PD-L1 expression has been associated with the response to immunotherapy in various cancers. Lin et al. reported a trial of LEP in patients with refractory BTC and found that positive PD-L1 expression in tumors pre-treatment was significantly associated with a higher clinical benefit rate and improved PFS and OS (20). In the present study, the subgroup of patients with positive PD-L1 expression achieved a higher ORR and had a significantly prolonged EFS and OS compared with patients who had negative PD-L1 expression, which was consistent with the study reported by Lin et al. Furthermore, patients with positive PD-L1 expression who underwent conversion resection had more tumor infiltrating lymphocytes clustered around the tumor. These results suggest that PD-L1 expression is a potential prognostic factor for the treatment of BTC with combined lenvatinib plus PD-1 inhibitors.

We evaluated the predictive value of TMB for response to combination therapy. Unexpectedly, using the median TMB as the cutoff value, patients with lower TMB exhibited a better objective response and longer OS compared with patients with higher TMB. Although these findings are contrary to many previous reports, similar trends have been found in some recent studies. For example, Wang et al. reported a phase II clinical trial of toripalimab in recurrent or metastatic nasopharyngeal carcinoma. They found that none of the patients with TMB value over 10 muts/Mb achieved an objective response to toripalimab and also had a short PFS (1.68-3.25 months) and OS (2.30-9.56 months) (31). Although our study found no statistically significant differences between the low- and high-TMB groups, there was a trend towards longer OS for patients with lower TMB and this is supported by previous research. However, it should be noted that these results may be due to selection bias. More research is required to confirm the objectivity of these results.

We attempted to identify genomic biomarkers for response to lenvatinib plus PD-1 inhibitors in patients with advanced BTC. WES of 29 patients demonstrated that DNAH17, SSPO or ARID1A alterations were significantly associated with poor response. Although there have been prior reports of abnormal expression of DNAH17 and SSPO genes in tumors, there is a lack of systematic research of the association between these abnormalities and outcomes of anti-tumor therapy. Despite this, the mutation of ARID1A in the present study was consistent with previous reports. Hu et al. found that loss of ARID1A activated Ang2-dependent angiogenesis and promoted hepatocellular carcinoma progression. In addition, ARID1A alterations are known to confer sensitivity to anti-angiogenic therapy (32). As a tumor suppressor gene, the impact of genomic amplification in ARID1A on anti-angiogenic function and immunotherapy requires further investigation in BTCs. We also identified P13K pathway mutations as another potential biomarker of prognosis. The association between hyperactivity and activation of the P13K pathway and response to radiotherapy and chemotherapy has been previously reported and is a known negative prognostic factor for various cancer types (33). Consistent with these previous findings, in our study, patients with mutations in the P13K pathway had worse outcomes than the wild type group. However, due to the heterogeneity of tumors, more studies in BTC are required to validate this result.

The key limitations of this trial include the following. (1) Five different PD-1 inhibitors were used and differences in drug mechanisms cannot be ignored. However, we found no significant differences in treatment efficiency between the different treatment regimens. (2) This trial had a non-randomized design with a relatively small number of patients enrolled, which may have led to participant bias and selection bias. Large-scale studies with long-term follow-up are needed to verify the effects of conversion therapy and discover the best biomarkers for predicting response. (3) Not all patients had enough pre-treatment biopsy tissue for PD-L1 and whole-genome sequencing, which limited the accuracy of tumor marker exploration.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/**Supplementary Material**.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the ethics committee of the Second Affiliated Hospital of Zhejiang University of Medicine. The patients/participants provided their written informed consent to participate in this study.

AUTHORS CONTRIBUTIONS

QZ and XL wrote the manuscript. LZ, YT, ZG, and MJ helped to collect patient data. SW provided the pathologic analysis. SY designed the research and supervised the report. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2021.751391/full#supplementary-material

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The Efficacy of TACE Combined With Lenvatinib Plus Sintilimab in Unresectable Hepatocellular Carcinoma: A Multicenter Retrospective Study

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Objective: To assess the efficacy and safety of transarterial Chemoembolization (TACE) combined with lenvatinib plus sintilimab in unresectable hepatocellular carcinoma (HCC).

Patients and Methods: The data of patients with unresectable HCC administered a combination therapy with TACE and lenvatinib plus sintilimab were retrospectively assessed. Patients received lenvatinib orally once daily 2 weeks before TACE, followed by sintilimab administration at 200 mg intravenously on day 1 of a 21-day therapeutic cycle after TACE. The primary endpoints were objective response rate (ORR) and duration of response (DOR) by the modified RECIST criteria.

Results: Median duration of follow-up was 12.5 months (95%CI 9.1 to 14.8 months). ORR was 46.7% (28/60). Median DOR in confirmed responders was 10.0 months (95%CI 9.0-11.0 months). Median progression-free survival (PFS) was 13.3 months (95%CI 11.9-14.7 months). Median overall survival (OS) was 23.6 months (95%CI 22.2-25.0 months).

Conclusions: TACE combined with lenvatinib plus sintilimab is a promising therapeutic regimen in unresectable hepatocellular carcinoma.

Keywords: hepatocellular carcinoma, transarterial chemoembolization, targeted therapy, immunotherapy, comprehensive therapy

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INTRODUCTION

Hepatocellular carcinoma (HCC) is one of the most prevalent malignant tumors and the fourth leading cause of cancer-related death worldwide (1). In patients with early-stage HCC, ablation, resection and transplantation have been recommended as curable therapies (2). Although unresectable disease may be treated by transarterial chemoembolization (TACE) and systemic therapy, most patients have a poor prognosis (3).

According to the Barcelona Clinic Liver Cancer (BCLC) staging and treatment strategy, TACE represents the standard treatment option for patients with intermediate stage HCC (4), being associated with longer survival compared with best supportive care (5). Meanwhile, only TACE hardly improves survival in advanced HCC (6, 7).

Lenvatinib has become a first–line systemic therapeutic option for advanced HCC (3, 4). In the phase III REFLECT trial (8), lenvatinib was non-inferior to sorafenib in overall survival (OS), showed greater objective response rate (ORR) and median progression-free survival (PFS), and conferred hepatic function in advanced HCC (9, 10).

Immune checkpoint inhibitors, as immunotherapeutic agents, have shown promising outcomes in patients with advanced HCC (11). In the KEYNOTE-240 study, although OS and PFS did not reach statistical significance, ORR and overall response were better than those of placebo, and some patients benefited from pembrolizumab (12). Sintilimab is a human immunoglobulin G4 (IgG4) monoclonal antibody that specifically binds to the PD-1 molecule on the surface of T cells, consequently blocking the tumor immune tolerance-inducing PD-1/programmed death-ligand 1 (PD-L1) pathway, re-activating the anti-tumor activities of lymphocytes, and inhibiting tumors. Sintilimab has been approved for marketing in December 2018, mainly for the treatment of recurrent or refractory classical Hodgkin lymphoma previously treated with at least two lines of chemotherapy. Sintilimab is also been applied for the treatment of various solid tumors in clinical practice, including lung cancer, liver cancer, and esophageal cancer, with notable safety and high efficacy.

Combination therapies have been researched for liver cancer, with favorable results (13), including PD-1 inhibitors plus lenvatinib, TACE plus Sorafenib and TACE plus Lenvatinib (14, 15). However, to date, TACE combined with lenvatinib plus sintilimab has not been studied for patients with unresectable HCC. Therefore, we conducted this retrospective study to assess the efficacy and safety of TACE combined with lenvatinib plus sintilimab in unresectable HCC.

PATIENTS AND METHODS

Patients

In this retrospective study, the medical records and imaging data of HCC patients were obtained at the Cancer Hospital of the University of Chinese Academy of Sciences, Tianjin Medical University Cancer Institute and Hospital, and First Hospital of Shanxi Medical University between January 2019 and March 2021. All patients were diagnosed with HCC by non-invasive criteria or biopsy. The non-invasive diagnostic criteria for HCC in patients with cirrhosis were: liver cirrhosis; tumor diameter larger than 1 cm based on four-phase multi-detector computed tomography (MDCT) or dynamic magnetic resonance imaging (MRI), and arterial hypervascularization with venous or delayed phase washout (16, 17). Inclusion criteria were: [1] BCLC B or C stage; [2] at least one measurable target lesion; [3] Eastern Cooperative Oncology Group Performance Status (ECOG-PS) score of 0-1; [4] Child-Pugh class A score of 5-6. Exclusion criteria were: [1] prior systemic therapy or immunotherapy; [2] follow up <6 months; [3] a history of autoimmune disease. This study was approved by the Ethics Committees of the Cancer Hospital of the University of Chinese Academy of Sciences, Tianjin Medical University Cancer Institute and Hospital, and First Hospital of Shanxi Medical University. All patients were required to provide written informed consent before inclusion in the study.

TACE

TACE was performed by interventional radiologists (F.C, J.Z, T.S, Y.C) with more than ten years of experience. After puncturing the femoral artery, celiac trunk and superior mesenteric artery angiography were performed selectively with a 5F catheter (RH catheter; Cook, Bloomington, Ind). When the tumor-feeding arteries were found, the catheter was advanced into them one by one; a 3F microcatheter (SP microcatheter; Terumo, Tokyo, Japan) was used for selective catheterization if necessary. Oxaliplatin (75 mg/m^2) was infused *via* the catheter, and iodized oil (Lipiodol Ultrafluido; Guerbet, Aulnay-sous-Bois, France) mixed with epirubicin (30-50 mg/m^2) was used to embolize tumor-feeding arteries. The TACE procedure was repeated 4-6 weeks later.

Systemic Therapy

Patients received lenvatinib at 12 mg (bodyweight >60 kg) or 8 mg (bodyweight < 60 kg) orally once daily 2 weeks before TACE (6). Patients were administered sintilimab at 200 mg intravenously on day 1 of a 21-day therapy cycle after the TACE procedure.

Follow-Up Visits

Follow-up visits were performed 4-6 weeks after the TACE procedure. The patients underwent chest CT, abdomen multiphase CT or MRI, and laboratory examinations during each follow-up visit. The laboratory examinations encompassed liver function tests, including bilirubin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), albumin (ALB) and γ-glutamyl transpeptidase (γ-GT) level assessment, along with prothrombin time (PT) and serum α-fetoprotein (AFP) level evaluation. OS was defined as the time from the first TACE treatment to death or the last follow-up. PFS was defined as the time from the first TACE treatment to disease recurrence or the last follow-up. Intrahepatic tumor progression (25% increase from baseline) and transient deterioration of liver function to Child-Pugh C, macrovascular invasion (MVI) or extrahepatic metastasis was considered to indicate disease progression (6).

Statistical Analysis

Primary endpoints were objective response rate (ORR) and duration of response (DOR) determined by the modified RECIST criteria. The Kaplan-Meier method was used to estimate DOR, PFS and OS. Patients with confirmed complete response (CR) or partial response (PR) were analyzed for DOR. All statistical analyses were performed with SPSS version 23.0.

RESULTS

Patient Demographics

Totally 60 patients were enrolled in the current study between January 2019 and March 2021. Six individuals were excluded because of prior sorafenib or lenvatinib or PD-1 treatment, and two were excluded for follow up <6 months; finally, 52 patients were analyzed (40 patients from Cancer Hospital of the University of Chinese Academy of Sciences, 6 patients from Tianjin Medical University Cancer Institute and Hospital, and 6 patients from First Hospital of Shanxi Medical University). Age, gender, Child-Pugh class, hepatitis B virus (HBV) infection ratio, alpha fetoprotein (AFP) levels, albumin-bilirubin (ALBI) score, BCLC stage and ECOG-PS score were examined. The baseline characteristics of the 60 patients were collected before therapy (**Table 1**).

Efficacy

Median duration of follow-up was 12.5 months (95%CI 9.1 to 14.8 months). Tumor assessments were based on the mRECIST criteria. Objective Response Rate (ORR) was 46.7% (28/60), with complete response (CR) and partial response (PR) observed in 4 and 24 patients, respectively. Twenty-three patients were rated as stable disease (SD), and nine had progressive disease (PD). Reductions of tumor size are shown in **Figure 1**. Median duration of response (DOR) for confirmed responders was 10.0 months (95%CI 9.0-11.0 months, **Figure 2**). Median progression-free survival (PFS) was 13.3 months (95%CI 11.9-14.7 months, **Figure 3**). Median overall survival (OS) was 23.6 months (95%CI 22.2-25.0 months, **Figure 4**). A patient was evaluated as CR after treating with TACE combined with lenvatinib plus sintilimab as shown in **Figures 5A1–5D2**.

Safety

Totally 44 patients (84.6%) showed adverse events (AEs) of any grade (**Table 2**). The most common treatment-related AEs were fatigue (30.8%), hypertension (25%), diarrhea (19.2%), decreased appetite (23%) and Palmar-plantar erythrodysesthesia (21.1%).

During the study, 48% of patients showed grade 3 AEs [n=25], with hypertension as the most common grade 3 event (24%); 5.7% of patients had grade 4 AEs [n=3]. Totally 3.8% of patients developed grade 5 AEs, including abnormal liver function (n=1) and alimentary tract hemorrhage (n=1). A total of 11.5% (n=6) of patients discontinued any treatment component because of adverse events; one patient died from treatment related alimentary tract hemorrhage on day 134.

DISCUSSION

Theraprutic options for unresectable HCC have been developed rapidly in recent years. Sorafenib was the only available systemic therapeutic over a decade ago, and lenvatinib has also become a first –line systemic therapeutic agent for advanced HCC after the REFLECT trial (18). Although immune checkpoint inhibitors alone do not achieve a very significant effect, the combination of immunotherapy and systemic therapy could be very satisfactory. In a phase Ib study, lenvatinib plus pembrolizumab showed promising results in patients with unresectable HCC (19). In this study the ORR reached 46.0% and 36.0% by the mRECIST and RECIST 1.1 criteria, respectively. Median DORs were 8.6 months by mRECIST and 12.6 months by RECIST v1.1. Median overall survival was 22 months. In the IMbrave150 trial (20), atezolizumab plus bevacizumab showed significantly better OS and PFS compared with sorafenib in patients with unresectable HCC. In this study, OS rates at 12 months were 67.2% and 54.6% with atezolizumab plus bevacizumab and sorafenib alone, respectively; median PFS times were 6.8 months and 4.3 months, respectively. Although the exact mechanism of this combination therapy is uncertain, it is possible that VEGF may play an important role in cancer immune evasion. VEGF can enhance immune-suppressive effects in the tumor microenvironment though 3 mechanisms (21), i.e., inhibition of DC maturation to reduce T-cell activation, reduction of T-cell tumor infiltration and increase of inhibitory cells such as

TABLE 1 | Baseline characteristics of the 52 patients.

Characteristic	NO.(%)
Age (years)	
≤65	40 (76.9%)
>65	12 (23.1%)
Gender	
Female	7 (13.6%)
Male	45 (86.4%)
HBV infection	
Yes	47 (90.4%)
No	5 (9.6%)
Child-Pugh score	
A	46 (88.5%)
B	6 (11.5%)
AFP (ng/mL)	
≤400	34 (65.4%)
>400	18 (34.6%)
ALBI score	
1	10 (19.2%)
2	40 (77.0%)
3	2 (3.8%)
ECOG- PS	
0	7 (13.5%)
1	45 (86.5%)
BCLC stage	
B	13 (25.0%)
C	39 (75.0%)
Macroscopic vascular invasion	19 (36.5%)
Extrahepatic site	21 (40.4%)
lung	10 (19.2%)
nodes	10 (19.2%)
bone	5 (9.6%)
other	3 (5.8%)

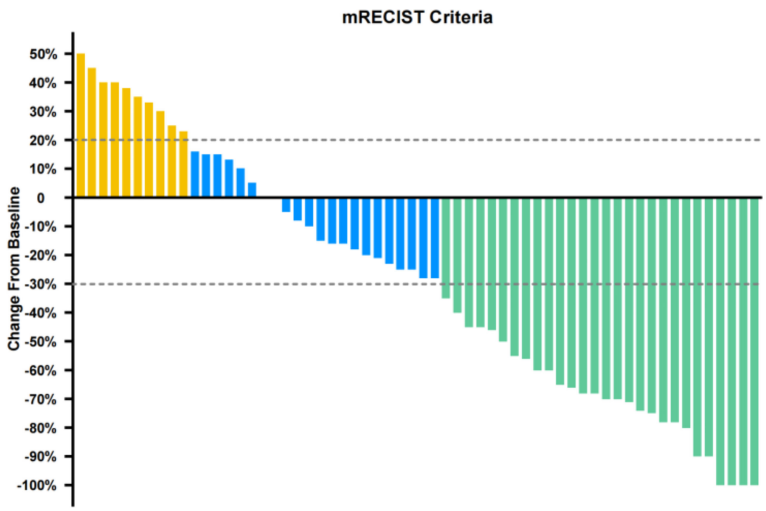

FIGURE 1 | Percentage changes from baseline (summed diameters of target tumors by mRECIST).

myeloid derived suppressor cells (MDSCs) and regulatory T cells (Tregs).

TACE is an effective treatment option for intermediate stage (multinodular, preserved liver function and ECOG PS=0) HCC (22–24). However, repeated TACE may lead to liver function impairment and even TACE resistance (25, 26), and TACE alone is unsatisfactory for patients in advanced stage (portal invasion or extrahepatic spread). Therefore, many studies have adopted TACE combined with systemic therapy for the treatment of unresectable HCC (27). TACE combined with sorafenib and lenvatinib, respectively, are commonly used in unresectable HCC (6, 28–30). The possible mechanism is that TACE induces angiogenesis and enhances the serum concentrations of VEGF because of local hypoxia, suggesting that VEGF may exert its greatest antiangiogenic effects before or after TACE (31). More importantly, recent studies found that pre-treatment with molecular targeted agents before TACE can normalize tumor vessels and upregulate VEGF, which may lead to a homogeneous distribution of lipiodol mixed anticancer drugs in the tumors (6). In this study, in order to obtain the best results, all patients received lenvatinib 2 weeks before TACE.

In present study, the ORR was 46.7% (28/60); CR and PR were observed in 4 and 24 patients, respectively. The DOR was 10.0 months (95%CI 9.0-11.0 months). PFS and OS were 13.3 months

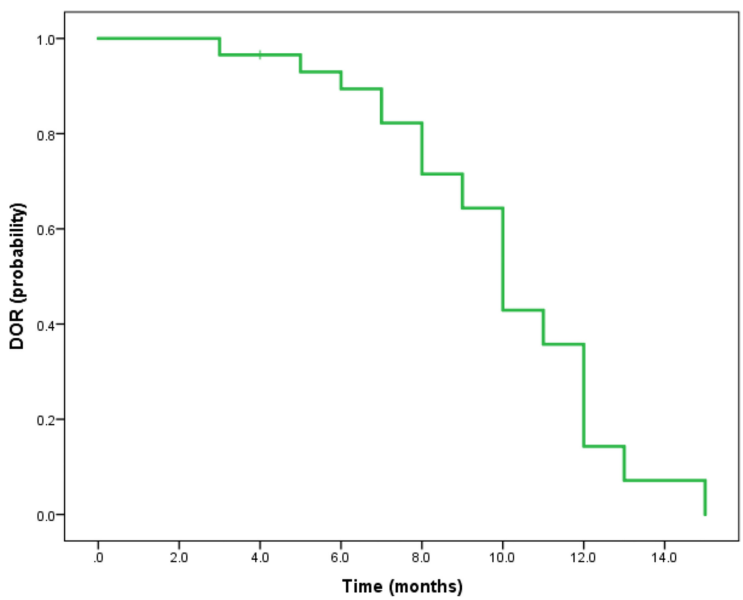

FIGURE 2 | Kaplan-Meier method-based estimate of DOR by mRECIST.

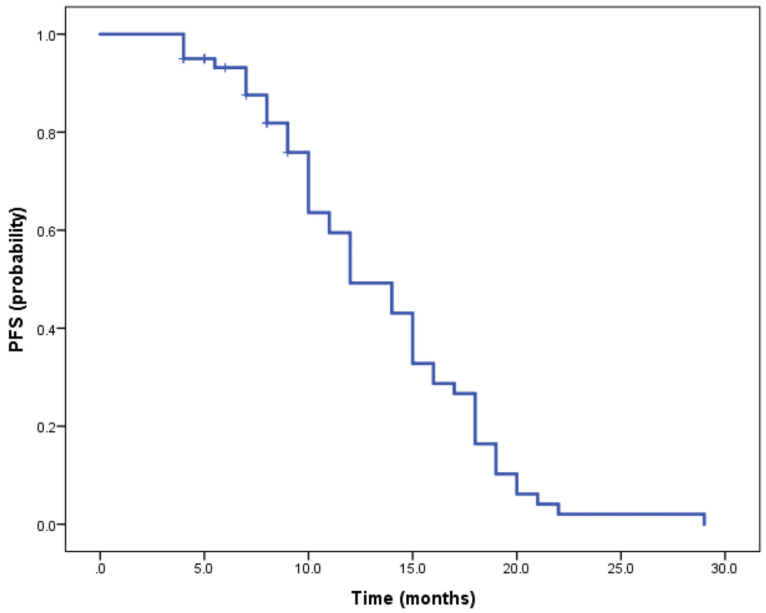

FIGURE 3 | Kaplan-Meier method-based estimate of PFS by mRECIST.

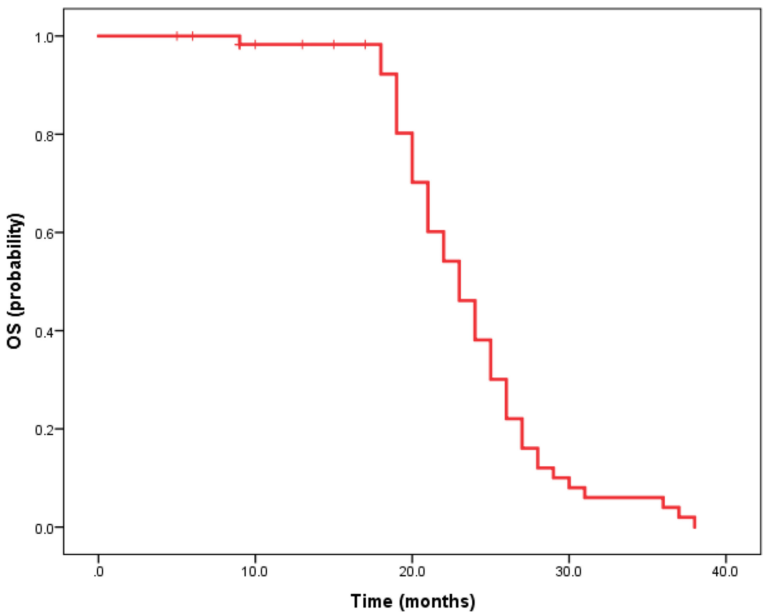

FIGURE 4 | Kaplan-Meier method-based estimate of OS by mRECIST. Imaging data of a 63-year-old male patient.

(95%CI 11.9-14.7 months and 23.6 months (95%CI 22.2-25.0 months), respectively. The median DOR, PFS and OS were longer than reported in previous trials combining PD-1 inhibitors and lenvatinib such as the IMbrave150 study (20) and other clinical trials (19). Therefore, this study showed that TACE combined with lenvatinib plus sintilimab is very effective in unresectable HCC.

This treatment was also safe as shown above. The most common treatment-related AEs were fatigue, hypertension, diarrhea, decreased appetite and Palmar-plantar erythrodysesthesia. In this study, 48%, 5.7% and 3.8% of patients had grade 3, 4 and 5 AEs, respectively. Totally 11.5% of patients discontinued any treatment component because of adverse events, and one individual died

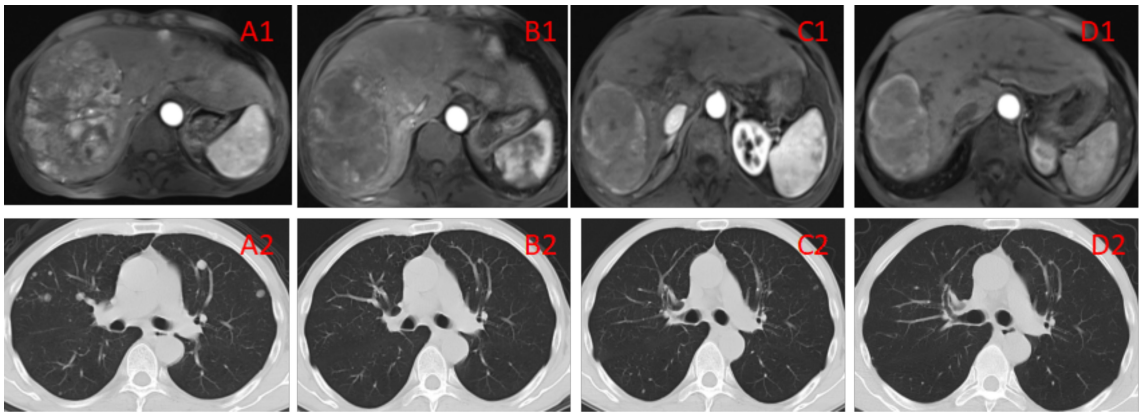

FIGURE 5A1–D2 | **(A1, A2)** Imaging manifestations of the patient before the treatment, showing a massive tumor accompanied by multiple small lesions in the right lobe of the liver, as well as multiple metastatic lesions in bilateral lungs. BCLC liver cancer stage: IIIB. **(B1, B2)** Imaging manifestations of the patient after 2 TACE sessions and 3 cycles of immunotherapy, showing the tumor in the right lobe of the liver with evident necrosis compared with the pretreatment condition as well as reduced number and sizes of multiple metastatic lesions in bilateral lungs. The efficacy evaluation showed PR. **(C1, C2)** Imaging manifestations of the patient after 4 TACE sessions and 6 cycles of immunotherapy, showing that the lesion in the right lobe of the liver was generally necrotic, as well as overtly reduced number of lesions in bilateral lungs. The efficacy evaluation showed PR. **(D1, D2)** Imaging manifestations of the patient after 12 cycles of immunotherapy, showing that the lesion in the liver was generally necrotic, with no lung lesions. The efficacy evaluation showed CR.

TABLE 2 | Treatment-related adverse effects.

AEs	Any Grade NO. (%)	Grade 1 NO. (%)	Grade 2 NO. (%)	Grade 3 NO. (%)	Grade 4 NO. (%)	Grade 5 NO. (%)
Fatigue	16 (30.8)	5	6	5	0	0
Hypertension	13 (25.0)	7	4	2	1	0
Diarrhea	10 (19.2)	5	3	2	0	0
Decreased appetite	12 (23.0)	4	5	3	0	0
Weight decreased	5 (9.6)	3	1	1	0	0
Palmar-plantar erythrodysesthesia syndrome	11 (21.1)	5	3	2	1	0
Proteinuria	2 (3.8)	0	0	1	1	0
Nausea	2 (3.8)	1	1	0	0	0
Thrombocytopenia	1 (1.9)	0	0	1	0	0
Abdominal pain	5 (9.6)	2	2	1	0	0
Hypothyroidism	5 (9.6)	2	2	1	0	0
Rash	4 (7.7)	1	3	0	0	0
Abnormal liver function	1 (1.9)	0	0	0	0	1
Alimentary tract hemorrhage	1 (1.9)	0	0	0	0	1

because of treatment related alimentary tract hemorrhage. The above results were comparable to those reported in previous studies examining combined treatments for unresectable HCC (19, 20, 32, 33), suggesting satisfactory safety and tolerability for this combination.

There were several limitations in this study. Firstly, this was a retrospective trial with a limited sample size, which may lead to potential bias. Besides, most patients in the current study had HBV infection, and prospective multicenter studies with other etiologies are required to validate these findings. Lastly, this was a one-arm study, without a control group. Randomized controlled trials of TACE combined with lenvatinib plus sintilimab versus lenvatinib plus sintilimab should be performed to confirm the efficacy and safety of this regimen.

In conclusion, the objective response rate, duration of response, progression-free survival and overall survival in this study were satisfactory, and adverse events were manageable. Therefore, TACE combined with lenvatinib plus sintilimab is very effective in unresectable HCC.

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

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

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Early Alpha-Fetoprotein Response Is Associated With Survival in Patients With HBV-Related Hepatocellular Carcinoma Receiving Lenvatinib

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Background/Purpose: Lenvatinib is a first-line treatment for unresectable hepatocellular carcinoma (uHCC). We assessed the value of early alpha-fetoprotein (AFP) response for predicting clinical outcomes with lenvatinib treatment in patients with HBV-related uHCC and elevated AFP levels.

Methods: This retrospective analysis included patients with HBV-related uHCC and baseline AFP levels ≥20 ng/ml who received lenvatinib for >1 month between November 2018 and May 2021. Early AFP response was defined as a >20% decrease in AFP serum level from baseline after 4 weeks of lenvatinib treatment. Radiological response (Response Evaluation Criteria in Solid Tumors v1.1), progression-free survival, and overall survival were assessed in AFP responders and non-responders.

Results: Of the 46 patients analyzed, 30 (65.2%) were early AFP responders and 16 (34.8%) were non-responders. Compared to the non-responders, early AFP responders had a significantly higher objective response rate (34.5% vs 6.3%, p=0.0349), disease control rate (82.8% vs 50.0%; p=0.0203) and longer median progression-free survival (13.0 vs 7.0 months; HR, 0.464; 95% CI, 0.222-0.967; p=0.028). A subsequent multivariate analysis confirmed that early AFP response (HR, 0.387; 95% CI, 0.183-0.992; p=0.0154), Eastern Cooperative Oncology Group Performance Status of 0 (HR, 0.890; 95% CI, 0.811-0.976; p=0.0132) and Albumin-Bilirubin grade 1 (HR, 0.457; 95% CI, 0.269-0.963; p=0.0327) were independent prognostic factors for longer progression-free survival.

Conclusion: AFP is an important prognostic factor and a predictive biomarker for survival benefit with lenvatinib treatment in patients with HBV-related uHCC.

Keywords: unresectable hepatocellular carcinoma, lenvatinib, AFP response, biomarker, targeted therapy, survival

INTRODUCTION

Worldwide, liver cancer is the sixth most commonly diagnosed cancer, with an estimated 905,677 new cases and 830,180 deaths in 2020 (1). The most common type of liver cancer is hepatocellular carcinoma (HCC), which accounts for 75–85% of cases (2). As HCC is asymptomatic until late in the natural disease course, most patients present with advanced disease, and fewer than 20% of patients are suitable candidates for radical surgery (3). Hence, systemic therapies are the mainstay of treatment for patients with HCC. Recently, systemic treatment options for HCC have expanded, with the emergence of new therapies, particularly targeted agents (4).

Lenvatinib is an oral multikinase inhibitor that targets vascular endothelial growth factor (VEGF) receptors 1-3, fibroblast growth factor (FGF) receptors 1-4, platelet-derived growth factor (PDGF) receptor-α, rearranged during transfection (RET), and KIT, with antiangiogenic and antiproliferative effects (5–7). Findings from clinical and real-world studies have consistently demonstrated the efficacy and good tolerability of lenvatinib in patients with unresectable HCC (uHCC) (8–11). In the REFLECT study, lenvatinib was the first drug to show noninferiority to sorafenib in patients with uHCC with respect to overall survival (OS), as well as statistically significant improvements in progression-free survival (PFS) and objective response rate (ORR) (12). Two thirds of patients in this study were from the Asia-Pacific region and half had hepatitis B virus-related HCC (12). Based on these results, lenvatinib was introduced into clinical practice as a new therapeutic option for the first-line treatment of uHCC (4, 13). However, there remains a need for non-invasive, convenient, and inexpensive biomarkers to assess treatment response and identify which patients are most likely to benefit from lenvatinib therapy.

Serum alpha-fetoprotein (AFP) is a glycoprotein that is overproduced in approximately 70% of patients with HCC (14). AFP levels can be measured using a simple blood test that is routinely available worldwide. Evaluation of changes in AFP levels over time can improve the performance of this biomarker versus a single assessment of the AFP level (15). The association of AFP response with radiological response and prognosis has been assessed in large cohorts of patients with HCC, including those treated with surgical resection, radiofrequency ablation, transarterial chemoembolization, cytotoxic chemotherapy and molecular targeted therapy, and can potentially guide clinical practice in the majority of cases (16–20). For example, previous studies of patients with HCC receiving sorafenib have shown that AFP response is associated with survival, despite differences in AFP criteria for study entry (>20 or >200 ng/ml) and AFP response definitions (21, 22). However, few studies have investigated the value of AFP as a biomarker in patients with advanced HCC treated with lenvatinib (23–25). In particular, a better understanding of the relationship between early AFP response and clinical outcomes in these patients may facilitate decisions on whether to continue lenvatinib treatment.

We performed a retrospective analysis to examine the association between early AFP response and treatment outcomes in patients with advanced HCC receiving lenvatinib in real-world clinical practice.

PATIENTS AND METHODS

Study Population

We retrospectively reviewed medical records of consecutive patients with HBV-related uHCC who received lenvatinib between November 2018 and May 2021 at the Cancer Center of the First Hospital of Jilin University. Inclusion criteria were: uHCC diagnosed using contrast enhanced computed tomography (CT), magnetic resonance imaging (MRI), or tumor biopsy; baseline AFP level ≥20 ng/ml; and lenvatinib treatment duration of ≥1 month. The study was conducted in accordance with the recently revised Declaration of Helsinki and approved by the ethics committee in our institution. Written informed consent was waived for this study because of the retrospective nature of the study. AFP assessment and definition of early AFP response

Serum AFP levels were measured at baseline (before the administration of lenvatinib) and at 1 month after starting lenvatinib therapy. There are no standardized cutoff values for AFP response, although thresholds of 20% and 50% change from baseline are frequently used (23, 24). In this study, early AFP response was defined as a >20% decrease in serum AFP levels after 1 month of lenvatinib treatment.

Treatment and Outcome Assessments

All patients received oral lenvatinib at a dose of 8 mg/day (bodyweight <60 kg) or 12 mg/day (bodyweight ≥60 kg). According to the instructions for lenvatinib administration, the dose was reduced or treatment was interrupted if grade ≥3 adverse events (AEs) or any unacceptable grade 2 AEs occurred. If a drug-related grade ≥3 AE or unacceptable grade 2 AE occurred, dose reduction or temporary interruption was maintained until the AE improved to grade 1 or 2. AEs were assessed using the National Cancer Institute Common Terminology Criteria for Adverse Events, version 4.0.

Radiological evaluations using enhanced CT or MRI were performed after 1 month of lenvatinib treatment and every 2-3 months thereafter, or whenever there was a sign or symptom suggesting tumor progression. Radiological response was determined according to the Response Evaluation Criteria in Solid Tumors (RECIST) v1.1. ORR was defined as the percentage of patients who achieved complete response (CR) or partial response (PR). The disease control rate (DCR) was defined as the percentage of patients who achieved a CR, PR or stable disease (SD). PFS was defined as the time from the start of lenvatinib treatment until tumor progression or death. OS was defined as the time from the start of lenvatinib treatment until death from any cause.

Statistical Analysis

Continuous variables were summarized using median values with interquartile (IQR) ranges, and intergroup values were compared using Mann–Whitney U tests. Categorical variables were summarized as number and percentage and were compared using a Fisher's exact test. PFS and OS were calculated using the Kaplan–Meier method and intergroup differences compared using a log–rank test. Radiological responses, PFS, and OS

were assessed in all patients and stratified by early AFP responders and AFP non-responders. Potential prognostic factors for PFS and OS were assessed using univariate and multivariate Cox proportional hazards models. All factors exhibiting a significant association with PFS or OS in the univariate analyses were included in the multivariate models. For subgroup analyses of PFS and OS, a univariate Cox proportional hazard model was used to estimate the hazard ratios (HRs) and corresponding 95% confidence intervals (CIs) for AFP responders versus AFP non-responders in specific patient subgroups. For all analyses, $p<0.05$ was considered statistically significant and all reported p values are two-sided. All statistical analyses were performed using R software version 3.6.3 (http://www.R-project.org/).

RESULTS

Patients

Medical records from a total of 79 patients were screened, of whom 46 were included in the analysis. Reasons for exclusion were diagnosis of non-HBV related HCC (n=4), baseline AFP <20 ng/ml (n=29). At baseline, more than half of patients (65.2%) were classified as Child-Pugh class A. Extrahepatic metastases were present in 52.2% of patients and portal vein thrombosis was noted in 34.8%. The median tumor size was 5.6 cm, with 17.4% and 80.4% of patients classified as Barcelona Clinic Liver Cancer (BCLC) stages B and C, respectively. In total, 82.6% of patients had received lenvatinib as first-line therapy, and the patients in the second-line setting are all progressed after sorafenib. The median follow-up period was 18.0 (IQR, 3-29) months. Overall, 10 (21.7%) patients had a lenvatinib dose reduction and three (6.5%) had a treatment interruption due to AEs.

Of the 46 patients analyzed, 30 (65.2%) were early AFP responders and 16 (34.8%) were AFP non-responders. Median baseline AFP levels were 660.25 ng/ml and 1199.0 ng/ml in early AFP responders and non-responders, respectively. Baseline characteristics were similar between early AFP responders and non-responders, except for age (p=0.0336), portal vein thrombosis (p=0.0205) and ascites(p=0.04) (**Table 1**).

Relationship Between Early AFP Response and Imaging Response

No patients achieved a CR. Among early AFP responders, PR, SD, and PD were observed in 10 (34.5%), 14 (48.3%), and 5 (17.2%) patients, respectively, compared with 1 (6.3%), 7 (43.8%), and 8 (50.0%) patients, respectively, in the non-responder group. Early AFP responders had a significantly higher ORR (34.5% vs 6.3%; p=0.0349) and DCR (82.8% vs 50.0%; p=0.0203) versus non-responders (**Table 2**).

Relationship Between Early AFP Response and Survival Outcome

Early AFP responders had a significantly longer median PFS compared with non-responders (13.0 vs 7.0 months; p=0.028; **Figure 1A**). The results of univariate and multivariate analyses for PFS are presented in **Table 3**. In the univariate analysis, patients were more likely to have longer PFS if they had an early AFP response (HR, 0.464; 95% CI, 0.222-0.967; p=0.0404), ECOG PS of 0 (HR, 0.431; 95% CI 0.265-0.897; p=0.0398) and ALBI grade 1 (HR, 0.538; 95% CI, 0.290-0.973; p=0.0462).A subsequent multivariate analysis confirmed that early AFP response (HR, 0.387; 95% CI, 0.183-0.992; p=0.0154), Eastern Cooperative Oncology Group Performance Status of 0 (HR, 0.890; 95% CI, 0.811-0.976; p=0.0132) and Albumin-Bilirubin grade 1 (HR, 0.457; 95% CI, 0.269-0.963; p=0.0327) were independent prognostic factors for longer progression-free survival.

Median OS was 17.0 months for early AFP responders compared with 12.0 months for non-responders (p=0.16; **Figure 1B**). The results of univariate and multivariate analyses for OS are presented in **Table 4**. In the univariate analysis, patients were more likely to have longer OS if they had ECOG PS of 0 (HR, 0.531; 95% CI, 0.324-0.991; p=0.0498) and ALBI grade 1 (HR, 0.434; 95% CI 0.258-0.855; p=0.0320). Multivariate analysis showed that ECOG PS of 0 (HR, 0.479; 95% CI, 0.314-0.876; p=0.0332), and ALBI grade 1 (HR, 0.551; 95% CI, 0.160-0.897; p=0.0346) were independent prognostic factors for longer OS.

Relationship Between Liver Function and Survival Outcome

We evaluated the association between AFP response and change in liver function. Deterioration of liver function was defined as a change from Child-Pugh class A to Child-Pugh class B after initiating lenvatinib treatment or, in the case of patients with Child-Pugh class B7, the patient's Child-Pugh score increased to ≥8 points. Most patients (31/46, 67.4%) showed maintained or improved liver function during lenvatinib therapy. There was no significant association between AFP response and change in liver function (p=0.42). Median PFS was significantly longer in patients with maintained or improved liver function compared with those whose liver function deteriorated during treatment (13.0 months vs 5.0 months; p=0.015; **Figure 2A**). Median OS was not reached in the maintained or improved liver function group and was 13 months in the deteriorated liver function group (p=0.081; **Figure 2B**).

DISCUSSION

Findings from this study in patients with uHCC receiving lenvatinib showed that early AFP responders achieved a significantly higher ORR and DCR compared with AFP non-responders. These results are consistent with previous studies of the relationship between early AFP decline and radiological response in patients with uHCC treated with lenvatinib (23–25). In one study, patients with a sustained reduction of AFP from 2 to 4

TABLE 1 | Baseline characteristics by AFP response.

	AFP response (N = 30)	AFP none response (N = 16)	p value
Gender, male/female			
Male	26 (86.7%)	16 (100%)	0.1264
Female	4 (13.3%)	0	
Age, ≥60/<60 years			
≥60	13 (43.3%)	2 (12.5%)	0.0336
<60years	17 (56.7%)	14 (87.5%)	
Baseline AFP,ng/mL			
n (nmiss)	30 (0)	16 (0)	
Median	660.25	1199.0	0.8717
AST,U/L			
n (nmiss)	30 (0)	16 (0)	
Median	48.50	33.15	0.1195
ALT,U/L			
n (nmiss)	30 (0)	16 (0)	
Median	40.40	26.75	0.0922
Total bilirubin, μmol/L			
n (nmiss)	30 (0)	16 (0)	
Median	22.95	19.45	0.4262
Albumin, g/L			
n (nmiss)	30 (0)	16 (0)	
Median	37.10	39.90	0.4745
Platelet count,$\times10^9$/L			
n (nmiss)	29 (1)	14 (2)	
Median	114.00	128.50	0.3643
Hemoglobin, g/L			
n (nmiss)	29 (1)	14 (2)	
Median	146.00	139.00	0.0656
Prothrombin time, s			
n (nmiss)	27 (3)	14 (2)	
Median	12.40	11.90	0.1645
International normalized ratio			
n (nmiss)	27 (3)	14 (2)	
Median	1.10	1.03	0.2207
BCLC stage B/C			
A	0	1 (6.3%)	0.1512
B	7 (23.3%)	1 (6.3%)	
C	23 (76.7%)	14 (87.5%)	
Maximum tumor diameter, cm			
n (nmiss)	30 (0)	16 (0)	
Median	6.00	3.60	0.1451
Number of tumors, solitary/multiple			
Null	2 (6.7%)	3 (18.8%)	0.451
solitary	8 (26.7%)	4 (25.0%)	
multiple	20 (66.7%)	9 (56.3%)	
Extrahepatic metastasis, yes/no			
Yes	14 (46.7%)	2 (12.5%)	0.0205
No	16 (53.3%)	14 (87.5%)	
Portal vein thrombosis, yes/no			
Yes	14 (46.7%)	2 (12.5%)	0.0205
No	16 (53.3%)	14 (87.5%)	
Cirrhosis, yes/no			
Yes	24 (80.0%)	9 (56.3%)	0.0884
No	6 (20.0%)	7 (43.8%)	
Ascites, yes/no			
Yes	17 (56.7%)	4 (25.0%)	0.04
No	13 (43.3%)	12 (75.0%)	
Lenvatinib as first line treatment, yes/no			
Yes	27 (90.0%)	11 (68.9%)	0.1459
No	3 (10.0%)	4 (25.0%)	
Unknown	0 (0)	1 (6.3%)	
ECOG PS 0/1,n (%)			
0	17 (56.7)	10 (62.5)	0.2903
1	13 (43.3)	6 (37.5)	

(Continued)

TABLE 1 | Continued

	AFP response (N = 30)	AFP none response (N = 16)	p value
ALBI grade 1/2			
<=-2.6	9 (30.0)	8 (50.0%)	0.1233
-2.6~-1.39	21 (70.0)	7 (43.8%)	
>-1.39		1 (6.3%)	
Child-Pugh class A/B			
A	21 (70.0%)	9 (56.2%)	0.2709
B	9 (30.0%)	7 (43.8%)	

ALBI, albumin-bilirubin; AFP, alpha-fetoprotein; BCLC, Barcelona Clinic Liver Cancer; ECOG PS, Eastern Cooperative Oncology Group performance status; SD, standard deviation.

TABLE 2 | Relationship between early AFP response and imaging response.

n (%)	Early AFP response (n = 29*)	AFP non-response (n = 16)	p value
Imaging response			
CR	0	0	
PR	10 (34.5)	1 (6.3)	
SD	14 (48.3)	7 (43.8)	
PD	5 (17.2)	8 (50.0)	
ORR			
Yes	10 (34.5)	1 (6.3)	0.0349
DCR			
Yes	24 (82.8)	8 (50.0)	0.0203

AFP, alpha-fetoprotein; CR, complete response; DCR, disease control rate; ORR, objective response rate; PD, progressive disease; PR, partial response; SD, stable disease.
**One patient lacked further imaging examination at data cut-off.*

weeks after lenvatinib initiation had a higher ORR compared with patients with a non-sustained reduction (67% vs 0%; p=0.02) (24). Another study reported that, among patients with baseline AFP ≥10 ng/ml, AFP responders (defined as those with an AFP reduction of ≥40%) versus non-responders had a significantly higher ORR (68.4% vs 7.1%; p<0.001) and DCR (84.2% vs 36.0%; p=0.009), and AFP response was the only significant predictor of objective response (odds ratio, 51.389; 95% CI, 4.888-540.281; p=0.001) (23). In addition, a multivariate analysis showed that a decrease in AFP level was an independent factor associated with response to lenvatinib (adjusted odds ratio, 10.3; 95% CI, 1.81-58.7; p<0.01) (25). Our results, together with this previous evidence, suggest that early AFP non-response can help to identify patients who are less likely to respond to lenvatinib and may therefore require more frequent imaging assessments.

Moreover, surgical resection remains the most important radical treatment for patients with liver cancer. For patients with advanced liver cancer who do not have an opportunity for surgery, transformational resection (resection after the cancer is reduced or downgraded) has a key role (26). From the perspective of transformational resection, the ORR is one of the most important considerations in the systemic treatment plan (26). However, efficacy predictors for the systemic treatment of liver cancer are lacking. Our research and previous studies have found that patients achieving an early AFP response have a significantly better tumor response compared with non-responders. Therefore, early AFP response may have value in guiding systemic treatment prior to transformational resection.

AFP response has previously been associated with survival in studies of patients with uHCC treated with drugs other than lenvatinib (21, 27). In patients treated with immune checkpoint inhibitors (nivolumab or pembrolizumab), early AFP response (>10% reduction in AFP within 4 weeks of treatment) predicted better objective response and survival (27). Early AFP response was also a significant independent predictor for better PFS and OS following antiangiogenic systemic therapy (21). Furthermore, similar results have been reported in patients receiving locoregional treatments, despite different AFP entry criteria and definitions of AFP response (28, 29). These previous reports support our findings that early AFP response may be a useful predictive marker for survival in patients with uHCC receiving lenvatinib, thereby helping to identify patients with a better prognosis and those who are candidates for other therapy options. The ability of AFP response to predict outcomes in uHCC may be explained by the role of AFP in promoting the growth, proliferation, and metastasis of HCC, and eliciting the escape of HCC from immune surveillance (14, 30, 31). Until now, no studies have investigated the value of AFP response in predicting the survival of patients with uHCC receiving lenvatinib. Our study demonstrated that early AFP responders achieved significantly longer PFS compared with non-responders. In addition, early AFP response was an independent prognostic factor for longer PFS in multivariate analysis. However, our results were inconclusive on whether early AFP response was predictive of OS, as the p-value was not significant in the univariate analysis(HR, 0.556; 95% CI, 0.240-1.288; p=0.1710). This may be related to the limited sample size and short follow-up time.

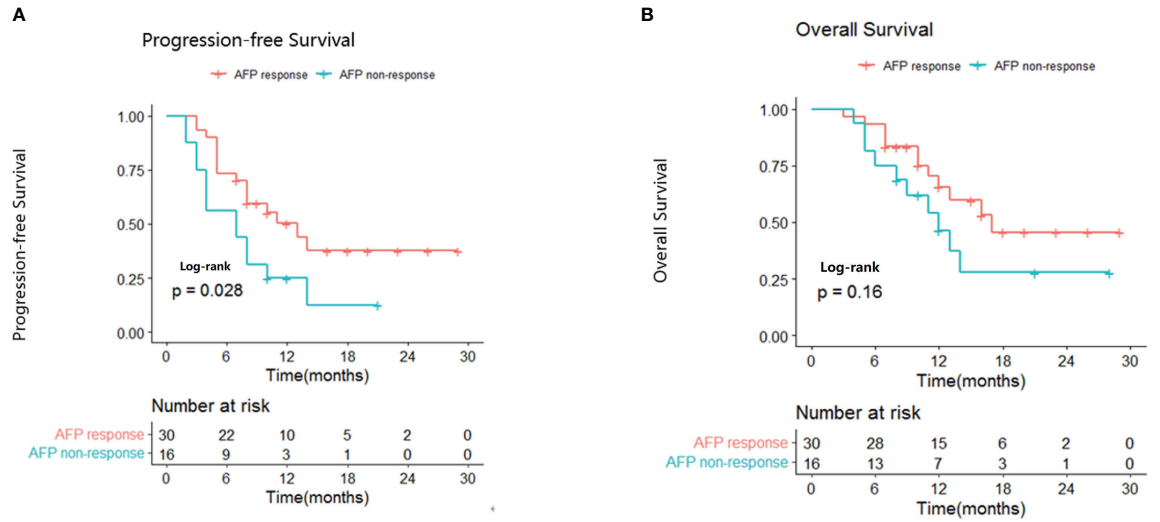

FIGURE 1 | Kaplan-Meier curves for progression-free survival **(A)** and overall survival **(B)** in AFP responders and non-responders.

TABLE 3 | Univariate and multivariate analysis for PFS.

	HR	95% CI	p value
Univariate analysis			
Age, ≥60/<60 years	0.781	0.355-1.718	0.5386
Early AFP response, yes/no	0.464	0.222-0.967	0.0404
Baseline AFP, ≥400/<400 ng/ml	1.414	0.681-2.935	0.3530
Ascites, yes/no	1.377	0.664-2.853	0.3897
Cirrhosis, yes/no	1.127	0.497-2.555	0.7746
Maximum tumor diameter, ≥10/<10 cm	0.420	0.143-1.235	0.1150
Number of tumors, solitary/multiple	0.793	0.332-1.893	0.6014
Extrahepatic metastasis, yes/no	1.585	0.633-3.972	0.3255
Portal vein thrombosis, yes/no	0.825	0.374-1.819	0.6338
BCLC stage, B/C	0.711	0.270-1.872	0.4903
ECOG PS, 0/1	0.431	0.265-0.897	0.0398
ALBI grade, 1/2	0.538	0.290-0.973	0.0462
Child-Pugh class, A/B	0.658	0.311-1.393	0.2742
Multivariate analysis			
Age, ≥60/<60 years	0.535	0.191-1.499	0.2341
Early AFP response, yes/no	0.387	0.183-0.992	0.0154
ECOG PS, 0/1	0.489	0.411-0.976	0.0132
Extrahepatic metastasis, yes/no	1.659	0.525-3.517	0.5271
Portal vein thrombosis, yes/no	1.059	0.437-2.324	0.6574
ALBI grade, 1/2	0.457	0.269-0.963	0.0327

ALBI, albumin-bilirubin; AFP, alpha-fetoprotein; BCLC, Barcelona Clinic Liver Cancer; CI, confidence interval; ECOG PS, Eastern Cooperative Oncology Group performance status; HR, hazard ratio.

Previous studies have shown that in some patients with grade 2 albumin-bilirubin at baseline, a decrease in tumor burden in response to treatment leads to an improvement in liver function (32). However, in our study, there was no significant association between AFP response and liver function, which may be related to patient selection. Patients with advanced HCC treated with lenvatinib have previously been shown to have maintained or improved liver functional reserves after 4 and 12 weeks (33). Consistent with this observation, approximately two thirds of patients in the present study had maintained or improved liver function during lenvatinib therapy. As expected, patients with stable liver function had better survival compared with those with worsening liver function.

This study has several limitations. Firstly, it was a retrospective study with a modest sample size and, consequently, the findings require confirmation in further studies with larger numbers of patients. Secondly, as only patients with elevated AFP levels (serum AFP ≥20 ng/ml) at the initiation of lenvatinib therapy were included, the results may not be applicable to patients whose baseline AFP levels are within the normal range. Thirdly, the

TABLE 4 | Univariate analysis for OS.

	HR	95% CI	p value
Univariate analysis			
Age, ≥60/<60 years	1.147	0.488-2.695	0.7537
Early AFP response, yes/no	0.556	0.240-1.288	0.1710
Baseline AFP, ≥400/<400 ng/ml	1.418	0.609-3.302	0.4183
Ascites, yes/no	1.743	0.744-4.086	0.2011
Cirrhosis, yes/no	1.248	0.486-3.203	0.6449
Maximum tumor diameter, ≥10/<10 cm	0.811	0.185-3.550	0.7808
Number of tumors, solitary/multiple	0.559	0.202-1.552	0.2646
Extrahepatic metastasis, yes/no	1.805	0.598-5.445	0.2946
Portal vein thrombosis, yes/no	0.941	0.380-2.326	0.8946
BCLC stage, B/C	0.517	0.152-1.763	0.2921
ECOG PS, 0/1	0.531	0.324-0.991	0.0498
ALBI grade, 1/2	0.434	0.258-0.855	0.0320
Child-Pugh class, A/B	0.718	0.301-1.711	0.4544
Multivariate analysis			
Age, ≥60/<60 years	0.900	0.295-2.746	0.8526
Early AFP response, yes/no	0.734	0.229-2.351	0.6023
ECOG, 0/1	0.479	0.314-0.876	0.0332
Extrahepatic metastasis, yes/no	1.934	0.952-3.751	0.2930
Portal vein thrombosis, yes/no	0.948	0.315-2.858	0.9246
ALBI grade, 1/2	0.551	0.160-0.897	0.0346

ALBI, albumin-bilirubin; AFP, alpha-fetoprotein; BCLC, Barcelona Clinic Liver Cancer; CI, confidence interval; ECOG PS, Eastern Cooperative Oncology Group performance status; HR, hazard ratio.

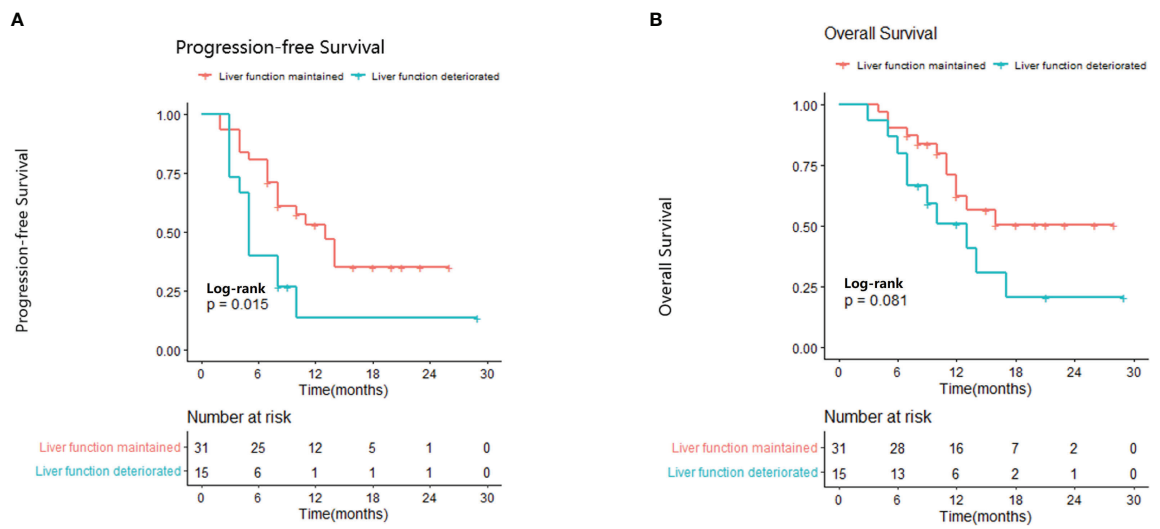

FIGURE 2 | Relationship between liver function and progression-free survival **(A)** and overall survival **(B)**.

etiology of HCC in this study was hepatitis B virus (HBV), with a higher proportion of patients with HBV infection than in previous studies conducted in Western countries. As HBV-related hepatitis or cirrhosis may contribute to elevation of AFP, further validation is needed.

In conclusion, early AFP response may be a useful predictor of better tumor response and longer PFS and OS in patients with uHCC receiving lenvatinib. Therefore, early AFP response should be taken into consideration when assessing treatment response to lenvatinib in patients with uHCC, particularly in those with elevated AFP levels prior to treatment initiation.

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 human participants were reviewed and approved by Ethics Committee of the First Hospital of Jilin

University Affiliation: First Hospital of Jilin University. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

BL and XS drafted the manuscript and contributed for the data analysis. J-YS, G-ZC, and XL critically revised the article. N-YW contributed for study concept and design. All authors read and approved the final manuscript, and all authors have taken due care to ensure the integrity of the work.

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Lenvatinib Plus Camrelizumab vs. Lenvatinib Monotherapy as First-Line Treatment for Unresectable Hepatocellular Carcinoma: A Multicenter Retrospective Cohort Study

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Background: Combining an antiangiogenic agent with an anti-PD-1 agent is a promising strategy for unresectable hepatocellular carcinoma (HCC).

Aims: To explore the effectiveness and tolerability of lenvatinib plus camrelizumab vs. lenvatinib monotherapy as a first-line treatment for unresectable HCC.

Methods: This multicenter, retrospective cohort study included patients with unresectable HCC treated with oral lenvatinib 8 mg daily and intravenous camrelizumab 200 mg every 3 weeks (L+C group) or lenvatinib 12 mg or 8 mg daily (L group) in four Chinese centers between September 2018 and February 2020. Tumor response was evaluated according to RECIST 1.1 and mRECIST. The outcomes included objective response rate (ORR), overall survival (OS), 1-year OS rate, progression-free survival (PFS), and safety.

Results: By March 31, 2021, 92 patients were finally included, with 48 and 44 in the L+C and L groups, respectively. ORR was significantly higher in the L+C group than in the L group (RECIST 1.1: 37.5% vs. 13.6%, P=0.009; mRECIST: 41.7% vs. 20.5%, P=0.029). Median OS and 95% confidence interval (CI) was 13.9 (13.3-18.3) months in the L group and not reached in the L+C group (P=0.015). The 1-year survival rate was 79.2% and 56.8% in the L+C and L groups, respectively. Median PFS was 10.3 (6.6-14.0) months and 7.5 (5.7-9.3) months in the L+C and L groups, respectively (P=0.0098). Combined therapy vs. monotherapy was independently associated with a prolonged OS (hazard ratio=0.380, 95% CI=: 0.196-0.739, P=0.004) and a prolonged PFS (hazard ratio=0.454, 95%CI=0.282-0.731, P=0.001). The safety profile was comparable between the two groups. The most common adverse event in the L+C and L groups was loss of appetite

(41.7% vs. 40.9%, P=0.941). Three patients in the L+C group and two in the L group terminated treatment owing to adverse events.

Conclusion: First-line lenvatinib plus camrelizumab showed better effectiveness than lenvatinib alone in patients with unresectable HCC.

Keywords: hepatocellular carcinoma, unresectable, lenvatinib, camrelizumab, objective response, survival

INTRODUCTION

Hepatocellular carcinoma (HCC) is a highly lethal invasive cancer arising in the liver (1, 2). The most important risk factors for HCC are infection with hepatitis B virus (HBV) or hepatitis C virus and/or preexisting liver cirrhosis (1–4). The worldwide age-standardized annual mortality rates for liver cancer are 13.9 per 100,000 men and 4.9 per 100,000 women (5, 6). HCC is typically asymptomatic throughout the initial clinical course of the disease (1, 4); hence about 50% of patients have advanced HCC at diagnosis (6). The 5-year overall survival (OS) of HCC is 18% for all stages, 31% for localized disease, 11% for regional disease, and only 2% for late-stage disease (5).

Although sorafenib has been used for many years as the first-line monotherapy for HCC, its use is associated with limited improvement in the prognosis of advanced HCC, and the newer option of lenvatinib provides better clinical benefits for patients with advanced HCC (7–10). The median progression-free survival (PFS) of patients with unresectable HCC treated with lenvatinib as a first-line monotherapy was 7.4 months, and the median OS was 13.6 months for lenvatinib compared with 12.3 months for sorafenib (7). Additionally, the objective response rate (ORR) was higher for lenvatinib than for sorafenib according to RECIST1.1 (24.1% vs. 9.2%) and mRECIST (40.6% vs. 12.4%) criteria. Nevertheless, further improvements in efficacy are required. Recent studies showed that a lenvatinib-based combination with immunotherapy could achieve better efficacy (11, 12). Combining antiangiogenic agents with immune checkpoint inhibitors has been a major breakthrough for the first-line treatment of HCC. Although atezolizumab plus bevacizumab as a first-line regimen for unresectable HCC resulted in better OS and PFS than treatment with sorafenib alone (13, 14), such combination therapy is quite expensive and not accessible to all patients.

In vitro studies have shown that lenvatinib and PD-1 inhibitors can exert synergistic antitumor effects, including activation of effector T cells and depletion of regulatory T cells in the tumor microenvironment, modulation of antigen-presenting cells and dendritic cell maturation, inhibition of immune-suppressive signaling, and normalization of tumor blood vessels (15–20). Furthermore, a retrospective analysis of first-line lenvatinib plus various PD-1 inhibitors in patients with unresectable HCC demonstrated tumor responses (21). A recent phase Ib study of lenvatinib plus pembrolizumab as first-line therapy for unresectable HCC provided preliminary evidence that combining an antiangiogenic agent with a PD-1 inhibitor exerted good antitumor activity against unresectable HCC (12). Another phase Ib study reported an ORR of 76.7% in patients with unresectable HCC treated with lenvatinib plus nivolumab (11). Lenvatinib is already covered by the medical insurance catalog for the treatment of HCC in China and has been widely applied in clinical practice. Therefore, studies are merited to investigate the effects of lenvatinib plus a PD-1 inhibitor as first-line therapy for patients with unresectable HCC.

Camrelizumab is a PD-1 inhibitor effective as a second-line treatment for HCC (22), and this agent has been approved for use in China. A retrospective study of patients with HCC who had received second-line therapy demonstrated that treatment with lenvatinib plus camrelizumab achieved longer survival than monotherapy with lenvatinib (23). Additionally, lenvatinib plus various PD-1 inhibitors with or without hepatic artery infusion chemotherapy (HAIC) was an effective first-line therapy for patients with advanced HCC (24). However, there remains no high-level evidence to guide drug selection among the available PD-1 inhibitors.

As mentioned above, the effectiveness and tolerability of lenvatinib plus camrelizumab as a first-line therapy still remain unclear. Therefore, this multicenter retrospective cohort study aimed to compare the therapeutic benefits and adverse reactions between lenvatinib plus camrelizumab and lenvatinib alone when given as a first-line treatment for patients with unresectable HCC.

MATERIALS AND METHODS

Study Design and Patients

This multicenter retrospective cohort study included patients with unresectable HCC from four study centers in China (**Supplementary Table 1**) between September 2018 and February 2020. The inclusion criteria were: 1) diagnosed with HCC according to the Guidelines for the Diagnosis and Treatment of Primary Liver Cancer in China (2019 edition) (25); 2) Barcelona Clinic Liver Cancer (BCLC) stage B or C; 3) received lenvatinib plus camrelizumab or lenvatinib monotherapy as the first-line therapy; 4) Child-Pugh class A or B; 5) Eastern Cooperative Oncology Group performance score (ECOG PS) of 0-2; and 6) at least one measurable lesion as defined by the Response Evaluation Criteria in Solid Tumors (RECIST) 1.1 and modified Response Evaluation Criteria in Solid Tumors (mRECIST) 1.1. The exclusion criteria were: 1) concomitant other primary malignant tumors; 2) incomplete clinical data; 3) severe comorbidities such as heart disease, severe renal dysfunction or infection; 4) uncontrolled hypertension; 5) had undergone major surgery or experienced gastrointestinal

hemorrhage within the previous 30 days; 6) pregnant or breastfeeding; 7) taking other antitumor agents; 8) total bilirubin >34.2 μmol/L, hepatic encephalopathy, or prolongation of prothrombin time (PT) >4 s; or 9) positive serology for hepatitis A, C or D or human immunodeficiency virus. This study was approved by the Ethics Committees of all four study centers. The requirement for individual informed consent was waived by the committees.

Treatment and Follow-Up

The patients were divided into the lenvatinib plus camrelizumab and the lenvatinib monotherapy groups. Patients in the lenvatinib monotherapy group received oral lenvatinib (Eisai, Co., Ltd., Tokyo, Japan) with the dosage adjusted according to body weight (12 mg for patients ≥60 kg and 8 mg for patients <60 kg, once per day). Patients in the lenvatinib plus camrelizumab group received oral lenvatinib 8 mg daily and intravenous camrelizumab 200 mg every 3 weeks (Hengrui Medicine Co., Ltd., Jiangsu, China).

The treatment was discontinued if intolerable adverse events (AEs) or disease progression occurred. If lenvatinib administration had to be interrupted due to AEs, camrelizumab was not used alone during the discontinuation of lenvatinib owing to the high incidence of reactive cutaneous capillary endothelial proliferation. If the AE was related to camrelizumab and was confirmed to be an immune-related AE, camrelizumab was interrupted if the AE was of grade 2 or permanently stopped if the AE was of grade 3 or higher. If causality could not be determined between lenvatinib or camrelizumab, the administration of both drugs was interrupted if the AE was of grade 2 or permanently stopped if the AE was of grade 3 or higher.

Routine blood, liver function, renal function, and coagulation function tests, measurement of α-fetoprotein (AFP) level, enhanced computed tomography (CT), or enhanced magnetic resonance imaging (MRI) of the upper abdomen were performed every 6-8 weeks.

Outcomes

The outcomes of this study included the ORR, disease control rate (DCR), OS, 1-year OS rate, PFS, and safety. An objective response was defined as a confirmed complete response (CR) or partial response (PR) according to RECIST and mRECIST 1.1. Disease control was defined as CR, PR, or stable disease (SD). The duration of treatment (DOT) was calculated. The time to response (TTR) was defined as the time from the start of treatment to the first confirmed CR or PR according to RECIST and mRECIST, respectively. OS was defined as the time from the start of treatment to death from any cause. PFS was defined as the time from the start of treatment to disease progression or death from any cause. The safety assessment included vital signs, hematological and biochemical laboratory tests, urinalysis, and electrocardiography. AEs were graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI CTCAE) version 4.03.

Statistical Analysis

All statistical analyses were performed using SPSS version 25.0 (IBM Corp., Armonk, NY, USA). Continuous data with a normal distribution are presented as means ± standard deviations and were compared using the independent t-test. Continuous data with a skewed distribution are presented as medians (ranges) and were analyzed with the Mann-Whitney U-test. Categorical data are presented as numbers (percentages) and were compared with the chi-squared test or Fisher's exact test. The Kaplan-Meier method was used to calculate the survival time and plot the curve, and the log-rank test was used to compare the two groups. Multivariable Cox regression was used to explore the factors related to OS and PFS, including therapy used, body mass index, ECOG PS, Child-Pugh class, AFP level, tumor number, BCLC stage, HBV infection, vascular invasion, intrahepatic metastasis, extrahepatic metastasis, hand-foot syndrome, hypertension, proteinuria, and dysphonia. The variables with P<0.10 in the univariable analyses were included in the multivariable analysis. Two-sided P-values <0.05 were considered statistically significant.

RESULTS

Study Population and Baseline Characteristics

Between September 2018 and February 2020, 113 patients with unresectable HCC in the four centers met the eligibility criteria (lenvatinib plus camrelizumab: n=58; lenvatinib monotherapy: n=55), but 21 patients were excluded. By the last follow-up on March 31, 2021, 92 patients were analyzed, of which 44 and 48 were in the lenvatinib plus camrelizumab and lenvatinib monotherapy groups, respectively (**Figure 1** and **Supplementary Table 1**). There were no significant differences between the two groups in the baseline clinical characteristics and previous treatments, including surgery and other treatments for HCC (all P>0.05; **Table 1**).

Effectiveness

In the lenvatinib plus camrelizumab group, four patients achieved CR, 16 patients achieved PR, 18 patients had SD, and 10 patients had progressive disease (PD). In the lenvatinib monotherapy group, two patients achieved CR, seven patients achieved PR, 24 patients had SD, and 11 patients had PD (**Table 2** and **Figure 2**). ORR was significantly higher in the lenvatinib plus camrelizumab group than in the lenvatinib monotherapy group (RECIST 1.1: 37.5% vs. 13.6%, P=0.009; mRECIST: 41.7% vs. 20.5%, P=0.029). The DCR was not significantly different between the two groups (RECIST 1.1: 75.0% vs. 75.0%, P>0.999; mRECIST: 79.2% vs. 75.0%, P=0.634; **Table 2**). The DOT was significantly longer in the lenvatinib plus camrelizumab group than in the lenvatinib monotherapy group (10.45 [7.25-15.47] months vs. 7.5 [5.1-11.35] months, P=0.009). The TTR was similar between the two groups (RECIST 1.1: 6.27 [4.13-7.43] vs. 4.13 [3.38-5.48], P=0.068; mRECIST: 4.13 [3.37-5.4] vs. 3.6 [2.08-4.61], P=0.172) (**Table 2**).

The median OS and 95% confidence interval (CI) was 13.9 (13.3-18.3) months in the lenvatinib monotherapy group, while the median OS was not reached in the lenvatinib plus

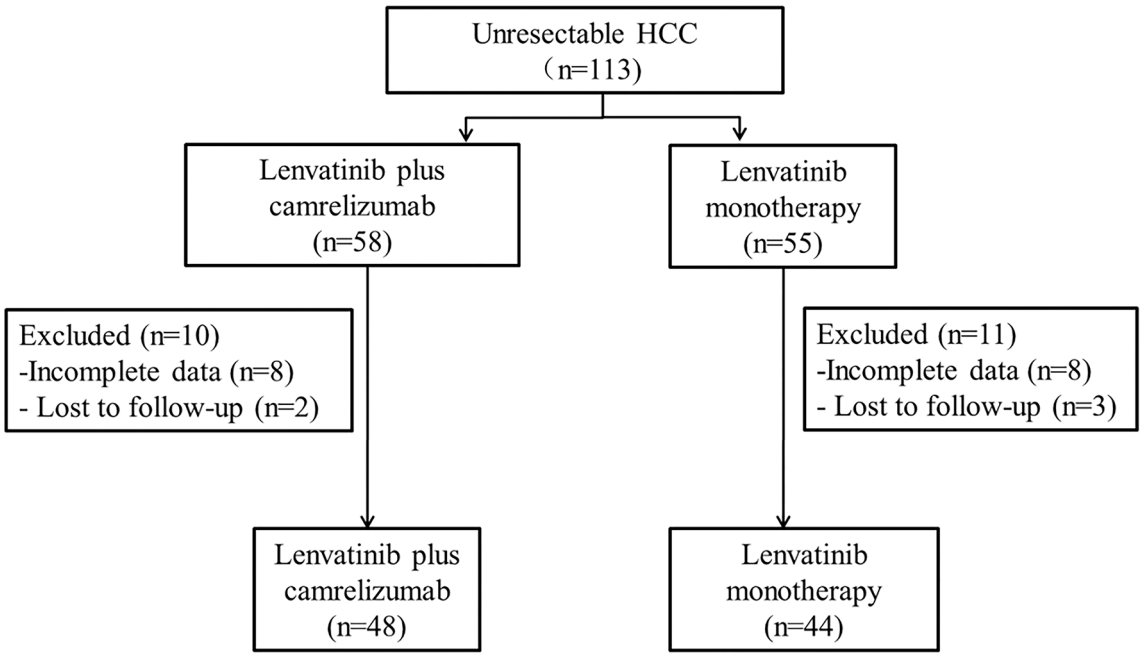

FIGURE 1 | Patient flowchart.

camrelizumab group (P=0.015; **Figure 3**). The 1-year survival rate was 79.2% in the lenvatinib plus camrelizumab group and 56.8% in the lenvatinib monotherapy group. The median PFS was significantly longer in the lenvatinib plus camrelizumab group than in the lenvatinib monotherapy group (10.3 [6.6-14.0] months vs. 7.5 [5.7-9.3] months, P=0.0098; **Figure 4**).

The results of the subgroup analyses are shown in **Figures 5** and **6**. Compared with lenvatinib alone, combination therapy was associated with a prolonged OS in males (HR=0.48, 95% CI: 0.24-0.91), in patients with Child-Pugh score ≤7 (HR=0.45, 95% CI: 0.23-0.90), in patients with >3 tumors (HR=0.46, 95% CI: 0.24-0.90), in patients with AFP >200 ng/mL (HR=0.37, 95% CI: 0.15-0.90), in HBV-positive patients (HR=0.48, 95% CI: 0.25-0.90), in patients with vascular invasion (HR=0.36, 95% CI: 0.18-0.73), and in patients without hypertension (HR=0.31, 95% CI: 0.16-0.62; **Figure 5**). There were no differences among subgroups for ECOG PS score, albumin-bilirubin (ALBI) score, intrahepatic metastasis, distant metastasis, hand-foot syndrome, and dysphonia. Subgroup analyses for the BCLC stage and urinary proteins could not be performed because of a lack of events in one subgroup each.

Compared with lenvatinib alone, combination therapy was associated with a prolonged PFS in males (HR=0.43, 95% CI: 0.26-0.71), in patients with Child-Pugh score ≤7 (HR=0.55, 95% CI: 0.34-0.88), in patients with >3 tumors (HR=0.50, 95% CI: 0.30-0.83), in patients with BCLC stage C (HR=0.43, 95% CI: 0.26-0.72), in patients with an ALBI score of -2.59 to -1.39 (HR=0.50, 95% CI: 0.27-0.91), in patients with AFP ≤200 ng/mL (HR=0.43, 95% CI: 0.22-0.83), in HBV-positive patients (HR=0.47, 95% CI: 0.29-0.77), in patients with vascular invasion (HR=0.42, 95% CI: 0.24-0.74), in patients with intrahepatic metastasis (HR=0.60, 95% CI: 0.36-0.99), in patients without distant metastasis (HR=0.39, 95% CI: 0.20-0.76), and in patients without hypertension (HR=0.52, 95% CI: 0.30-0.91; **Figure 6**). There were no differences among subgroups for ECOG, hand-foot syndrome and dysphonia.

Adverse Events

In the lenvatinib plus camrelizumab group, five patients (10.4%) had treatment-related AEs (TRAEs) leading to dose reduction, seven patients (14.6%) had TRAEs causing treatment suspension and three patients (6.3%) had TRAEs causing permanent termination of treatment. In the lenvatinib monotherapy group, six patients (13.6%) had TRAEs causing dose reduction, eight patients (18.2%) had TRAEs leading to treatment suspension, and two patients (4.5%) had TRAEs causing permanent termination of treatment.

The AEs which occurred in more than 20% of patients in the lenvatinib plus camrelizumab and lenvatinib monotherapy groups were hand-foot syndrome (22.9% vs. 25.0%, P=0.815), hypertension (33.3% vs. 38.6%, P=0.596), diarrhea (31.2% vs. 31.8%, P=0.953), loss of appetite (41.7% vs. 40.9%, P=0.941), proteinuria (29.2% vs. 34.1%, P=0.612) and increased alanine transaminase (22.9% vs. 25.0%, P=0.815). There were no statistically significant differences between the two groups in the incidences of any AEs (**Table 3**). The most common grade ≥3 AEs were hypertension (12.5% vs. 13.6%, P=0.872), proteinuria (4.2% vs. 4.5%, P=0.658), dysphonia (2.1% vs. 4.5%, P=0.467), diarrhea (2.1% vs. 2.3%, P=0.731), and increased ALT (2.1% vs. 2.3%, P=0.731).

TABLE 1 | Characteristics of the patients.

Characteristics	Lenvatinib plus camrelizumab group (n = 48)	Lenvatinib monotherapy group (n = 44)	P
Age (years)	53.81 ± 15.75	54.86 ± 18.25	0.692
Sex, male, n (%)	43 (89.6)	40 (90.9)	0.831
BMI (kg/m^2)	22.33 ± 2.89	22.66 ± 3.09	0.600
ECOG PS, n (%)			0.984
0	20 (41.7)	18 (40.9)	
1	21 (43.8)	19 (43.2)	
2	7 (14.6)	7 (15.9)	
Platelets (×10^9/L)	193.60 ± 86.13	187.77 ± 81.80	0.740
Total bilirubin (μmol/L)	18.72 ± 9.21	18.91 ± 8.89	0.920
Albumin (g/L)	36.79 ± 6.91	37.70 ± 6.45	0.504
Child-Pugh score, n (%)			0.752
≤7	41 (85.4)	40 (90.9)	0.417
>7	7 (14.6)	4 (9.1)	
AFP, n (%)			0.513
≤200 ng/mL	24 (50.0)	25 (56.8)	
>200 ng/mL	24 (50.0)	19 (43.2)	
Maximal diameter of tumor (cm)	9.95 ± 6.9	9.28 ± 4.6	0.508
Number of tumors, n (%)			0.638
≤3	9 (18.8)	10 (22.7)	
>3	39 (81.2)	34 (77.3)	
BCLC stage, n (%)			0.639
B	6 (12.5)	7 (15.9)	
C	42 (87.5)	37 (84.1)	
Vascular cancerous emboli, n (%)	36 (75)	34 (77.3)	0.799
Intrahepatic metastasis, n (%)	37 (80.4)	34 (77.3)	0.713
Distant metastasis, n (%)	21 (43.8)	19 (43.2)	0.956
ALBI, n (%)			0.707
1	16 (33.3)	17 (38.6)	
2	28 (58.3)	25 (56.8)	
3	4 (8.3)	2 (4.5)	
HBV infection, n (%)	41 (85.4)	38 (86.4)	0.896
Received previous treatment for HCC or not			0.452
Yes	32 (66.7%)	26 (59.1%)	
No	16 (33.3%)	18 (40.9%)	
Previous treatment(s) for HCC			
Surgery	11 (22.9%)	10 (22.7%)	0.983
Ablation	10 (20.1%)	9 (20.5%)	0.964
TACE or TAE	14 (29.2%)	10 (22.7%)	0.482

BMI, body mass index; ECOG PS, Eastern Cooperative Oncology Group performance status; AFP, α-fetoprotein; BCLC, Barcelona Clinic Liver Cancer; ALBI, assessment of the albumin-bilirubin; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; TAE, transarterial embolization; TACE, transarterial chemoembolization.

Multivariable Analysis of Factors Associated With OS and PFS

Cox regression analysis showed that combination therapy vs. monotherapy (HR=0.380, 95% CI: 0.196-0.739, P=0.004), ECOG PS 2 vs. 0 (HR=6.769, 95% CI: 2.183-20.989, P=0.001), hypertension (HR=0.393, 95% CI: 0.163-0.944, P=0.037), proteinuria (HR=0.196, 95% CI: 0.054-0.704, P=0.012), and dysphonia (HR=2.386, 95% CI: 1.022-5.57, P=0.044) were independently associated with a prolonged OS. (**Table 4**). Furthermore, combination therapy vs. monotherapy (HR=0.454, 95% CI: 0.282-0.731, P=0.001) and ECOG PS 2 vs. 0 (HR=2.955, 95% CI: 1.416-6.166, P=0.004) were independently associated with a prolonged PFS (**Table 5**).

DISCUSSION

This multicenter retrospective cohort study compared treatment responses and adverse events between lenvatinib plus camrelizumab and lenvatinib alone, given as the first-line treatment for unresectable HCC. The findings suggest that treatment with the combination of lenvatinib and camrelizumab might improve the ORR, PFS, and OS of patients when compared with lenvatinib monotherapy. The toxicity profile and tolerance were similar between the two groups, and no new safety signals were identified.

The combination of lenvatinib with a PD-1 inhibitor has been used in various solid cancers (26, 27), including HCC (12, 28–30), cholangiocarcinoma (31, 32), renal cancer (33–37), endometrial cancer (38–41), gastric cancer (42, 43) and adrenal cortical carcinoma (44). Lenvatinib plus a PD-1 inhibitor appears to be effective in patients with corresponding molecular subtypes regardless of the type of cancer, and the evidence from clinical trials indicates that the effectiveness of this treatment regimen might depend on the molecular subtype rather than the type of cancer. Hence, lenvatinib combined with an anti-PD-1 agent might be a promising option for many solid tumors.

The OS, PFS, and ORR (mRECIST) for patients in the lenvatinib monotherapy group were 13.9 months, 7.5 months,

TABLE 2 | Treatment effects.

N (%)	RECIST 1.1			mRECIST		
	Lenvatinib plus camrelizumab (n = 48)	Lenvatinib monotherapy (n = 44)	P	Lenvatinib plus camrelizumab (n = 48)	Lenvatinib monotherapy (n = 44)	P
CR	2 (4.2%)	2 (4.5%)	0.031	4 (8.3%)	2 (4.5%)	0.177
PR	16 (33.3%)	4 (9.1%)		16 (33.3%)	7 (15.9%)	
SD	18 (37.5%)	27 (61.4%)		18 (37.5%)	24 (54.5%)	
PD	12 (25.0%)	11 (25.0%)		10 (20.8%)	11 (25.0%)	
ORR	18 (37.5%)	6 (13.6%)	0.009	20 (41.7%)	9 (20.5%)	0.029
DCR	36 (75.0%)	33 (75.0%)	>0.999	38 (79.2%)	33 (75.0%)	0.634
DOT	10.45 (7.25-15.47)	7.5 (5.1-11.35)	0.009			
TTR	6.27 (4.13-7.43)	4.13 (3.38-5.48)	0.068	4.13 (3.37-5.4)	3.6 (2.08-4.61)	0.172

Data are expressed as frequency (percentage). RECIST, Response Evaluation Criteria in Solid Tumor; mRECIST, modified Response Evaluation Criteria in Solid Tumor; CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease; ORR, objective response rate; DCR, disease control rate; DOT, duration of treatment; TTR, time to response.

and 20.5%, respectively, in agreement with a previous study that reported corresponding values of 13.6 months, 7.4 months, and 24.1%, respectively, in patients with unresectable HCC (7). The median PFS for patients in the lenvatinib plus camrelizumab group was 10.3 months, the ORR (mRECIST) was 41.7%, and data for the estimation of OS were immature, indicating that the overall treatment benefits were greater in the lenvatinib plus camrelizumab group than in the lenvatinib monotherapy group. Our findings are supported by Wei et al. (23), who reported higher ORR and DCR for lenvatinib plus camrelizumab than for lenvatinib alone when used as second-line therapy. Additionally, a previous case report presented a patient with gastric cancer and liver metastasis who remained progression-free after 14 months of treatment with lenvatinib and camrelizumab (45). The above results may be associated with synergistic effects between the two types of immunotherapy, as suggested by *in vitro* experiments (15, 16), a retrospective study of lenvatinib combined with various anti-PD-1 therapies (21), and a phase Ib clinical trial (12). The exact mechanisms underlying this synergy remain uncharacterized. Besides its antiangiogenic actions, lenvatinib also modulates the immune system and reverses immunosuppression by promoting dendritic cell maturation, increasing the proliferation, tumor infiltration, and antitumor activity of effector T cells, upregulating T cell-related chemokines in the tumor, reducing the number of regulatory T cells, and inhibiting myeloid-derived suppressor cells (17). In the context of immune upregulation, inhibiting immune checkpoints might strengthen antitumor immunity (46, 47). Indeed, anti-PD-1 agents enhance tumor infiltration by dendritic cells and effector T cells (18), which would augment similar actions exerted by lenvatinib. Furthermore, inhibition of CTLA-4, another immune checkpoint, depletes regulatory T cells and thus reduces the degree of immunosuppression in the tumor microenvironment (19). Interestingly, the combination of lenvatinib with a PD-1 inhibitor attenuate immunosuppressive mechanisms and create an immune-active microenvironment substantially, and these effects were greater than those seen for each agent alone (18). Additionally, combining an inhibitor of vascular endothelial growth factor with a checkpoint inhibitor improved the migration of antigen-specific T cells (20). It has been reported that the use of multi-targeted tyrosine kinase inhibitor regorafenib combined with anti-PD-1 therapy in HCC could have a synergistic antitumor effect that is worth exploring, since regorafenib might modulate macrophage polarization, increase T cell activation, and thus enhance the efficacy of anti-PD-1 therapy (48). The concept of synergism is further supported by a meta-analysis concluding that lenvatinib plus

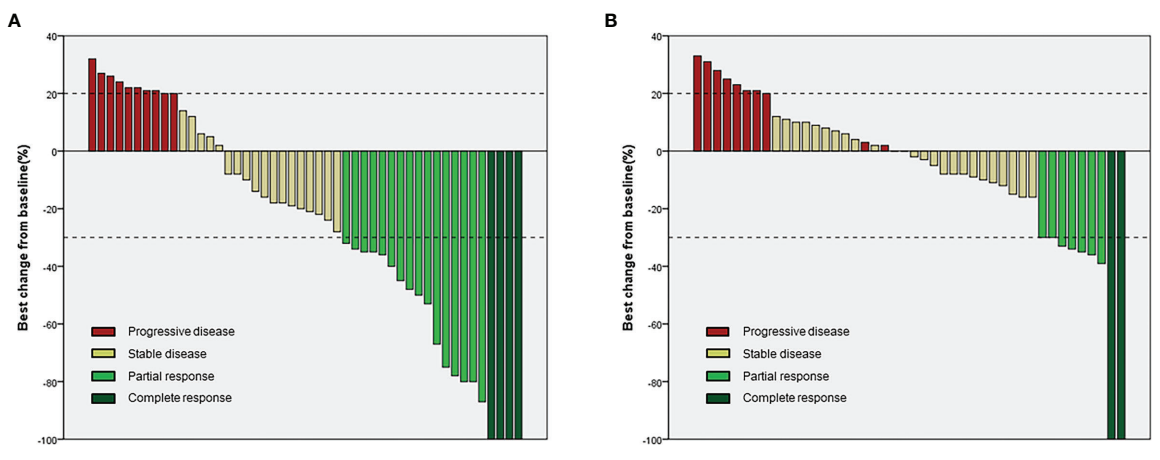

FIGURE 2 | Waterfall plots for the two groups. **(A)** Lenvatinib plus camrelizumab; **(B)** Lenvatinib.

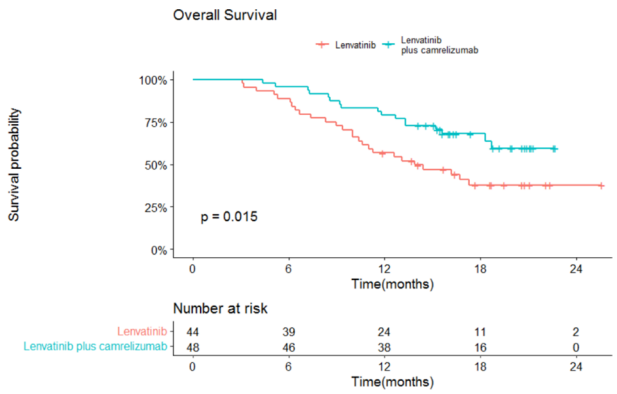

FIGURE 3 | Overall survival (OS) in the two groups.

pembrolizumab achieved better treatment outcomes than lenvatinib alone or pembrolizumab alone (49). Such combinations might act on both the vasculature and the stimulation of the antitumor immunity. Still, trials will have to examine these combinations.

Prior clinical investigations have suggested that the response to lenvatinib was smaller in patients with a high disease burden (50) or impaired liver function (51). One of the strengths of the present study is that it included many patients with late-stage liver cancer and thus reflects the situation encountered in real-world clinical practice. Thus, our investigation has a notable advantage over previous studies of lenvatinib as first-line therapy for HCC, including fewer patients with late-stage HCC. For example, the REFLECT trial, which compared lenvatinib monotherapy with sorafenib monotherapy, excluded patients categorized with Child-Pugh class B and ECOG PS score of 2 (7), whereas our study included such patients. Similarly, a recent retrospective analysis of 41 patients with advanced HCC included only one patient with an ECOG PS score of ≥1, and extrahepatic metastasis was present in only 24% of cases (52), compared with 43% in our study. Notably, a retrospective study

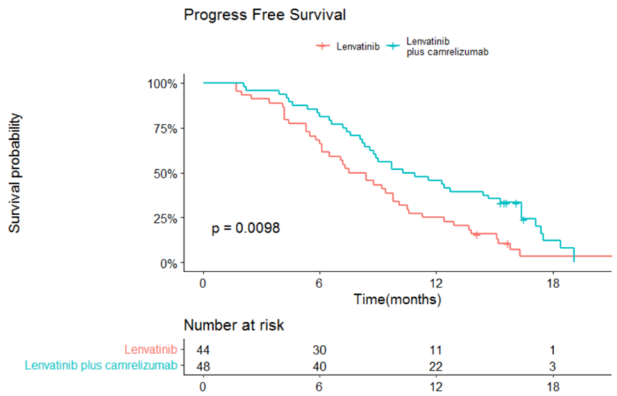

FIGURE 4 | Progression-free survival (PFS) in the two groups.

of patients not meeting the REFLECT trial eligibility criteria concluded that the efficacy of lenvatinib was comparable between patients with/without Child-Pugh class B and between patients with/without tumor in ≥50% of the liver (53), suggesting that lenvatinib remains effective in those with more advanced disease. Similarly, our study revealed very promising results for OS, PFS, and ORR in patients treated with combination therapy despite including many cases with late-stage HCC. Hence, our findings provide indirect evidence that first-line treatment with lenvatinib and camrelizumab might benefit patients with unresectable HCC in a real-world clinical setting.

Targeted immunotherapy greatly improves the ORR of advanced HCC. Combining anti-angiogenic drugs with immunotherapy for advanced or unresectable HCC can achieve an ORR of about 30%, and the median survival time of the patients can be increased to about 20 months (12, 13). By comparison, the median postoperative survival is only 12-15 months when surgical treatment is considered the first choice for HCC with resectable intrahepatic lesions and vascular invasion (i.e., technically resectable CNLC stage IIIa disease) (54). With the progress of drug treatment, many investigators began to explore the combination of target therapy and immunotherapy to reduce the tumor load for CNLC stage IIB and IIIA HCC, improve the R0 resection rate, and reduce the surgical risk, or resect the tumor after downstaging, to achieve better survival benefits than other treatments. Still, it is a retrospective cohort study with a small sample size (55). However, postoperative recurrence of HCC remains a major problem. Although the short-term remission rate was improved in patients with HCC who underwent surgical resection after targeted therapy and immunotherapy, relevant data about long-term OS are still lacking. Further research is needed to determine the optimal combination of drugs and the optimal time for surgical resection, develop methods of predicting the efficacy of combination therapy, and establish whether adjuvant therapy is necessary after surgical resection. As a result, many clinicians and patients still adopt a "wait-and-see" approach regarding combination therapy.

The incidence of AEs was relatively high in our study, but most AEs were manageable. The combined therapy did not significantly aggravate the incidence or severity of AEs compared with lenvatinib alone. The AEs were similar to those already reported for the two drugs (7, 12, 21–23), and no new safety signals were identified in this study. The treatments were well tolerated, with no grade 5 TRAEs. In agreement with our findings, a previous meta-analysis also showed that lenvatinib plus pembrolizumab had a similar safety profile to lenvatinib alone or pembrolizumab alone (49).

Beneficial clinical effects of transcatheter arterial chemoembolization (TACE) and HAIC have been demonstrated in patients with intermediate-stage liver cancer, but these treatment options are not suitable for those with late-stage disease (56, 57). The Chinese clinical guidelines for managing HCC recommend that patients with CNLC stage IIb/IIIa disease and some with stage IIIb disease are suitable for TACE and HAIC (58). However, there is evidence that multiple TACE procedures can cause an attenuation of the response and impairment of liver function (56). Interestingly,

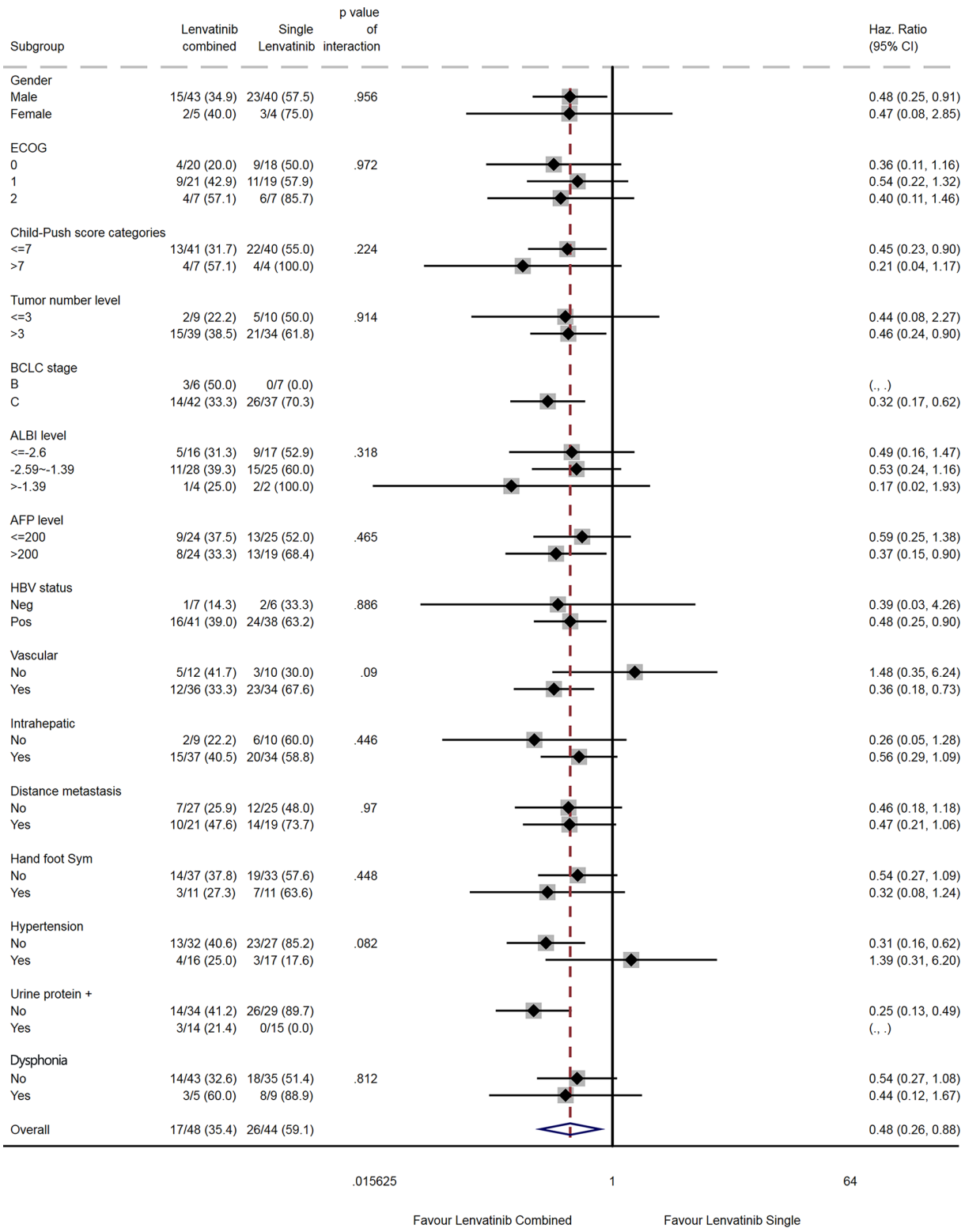

FIGURE 5 | Subgroup analysis of overall survival.

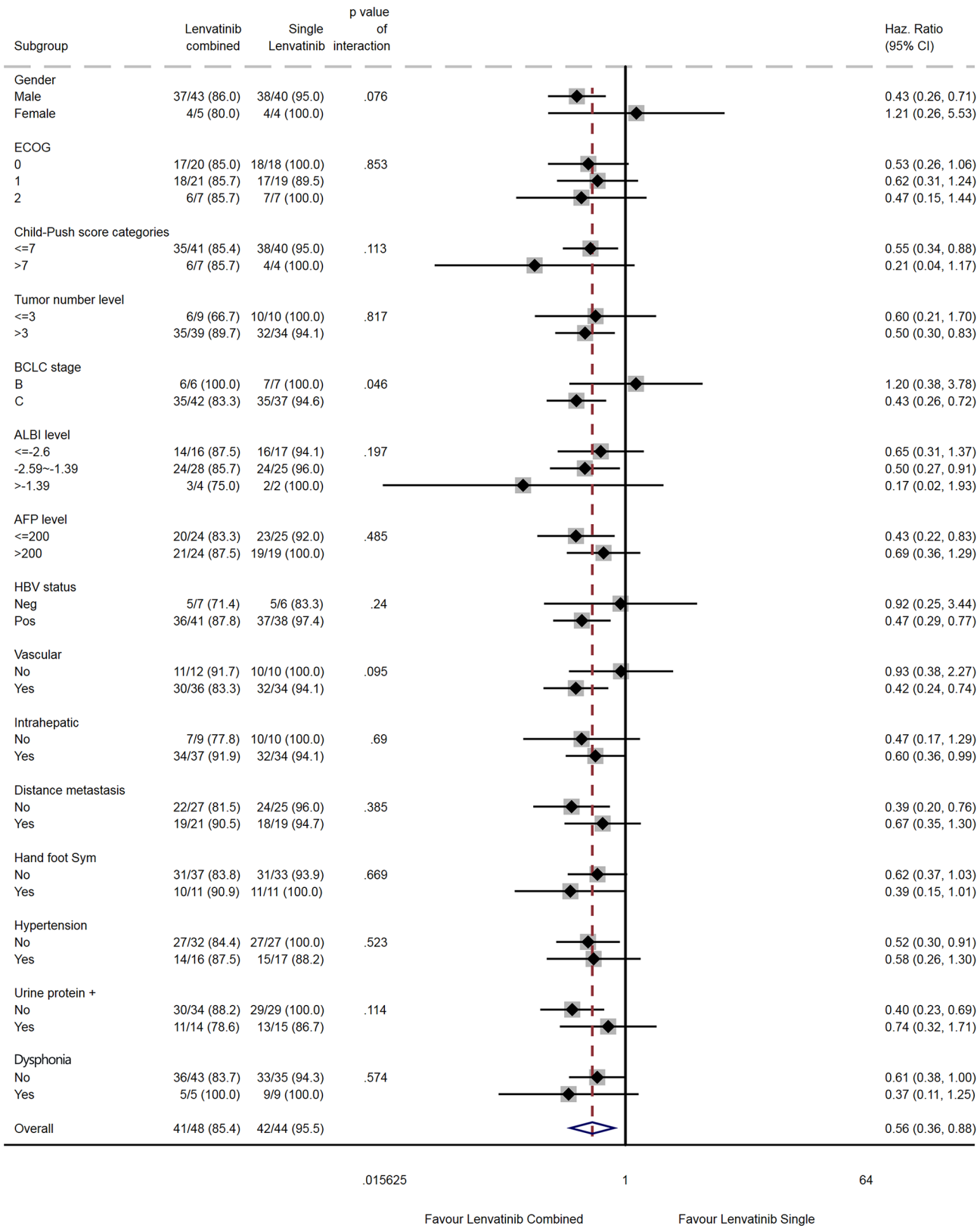

FIGURE 6 | Subgroup analysis of progression-free survival.

TABLE 3 | Adverse events of all grades and grade ≥3 in this study.

AE, n (%)	All grades			Grade ≥3		
	Lenvatinib plus camrelizumab (n = 48)	Lenvatinib monotherapy (n = 44)	P	Lenvatinib plus camrelizumab (n = 48)	Lenvatinib monotherapy (n = 44)	P
Hand-foot syndrome	11 (22.9%)	11 (25.0%)	0.815	0	1 (2.3%)	0.478
Hypertension	16 (33.3%)	17 (38.6%)	0.596	6 (12.5%)	6 (13.6%)	0.872
Diarrhea	15 (31.2%)	14 (31.8%)	0.953	1 (2.1%)	1 (2.3%)	0.731
Loss of appetite	20 (41.7%)	18 (40.9%)	0.941	0	1 (2.3%)	0.478
Proteinuria	14 (29.2%)	15 (34.1%)	0.612	2 (4.2%)	2 (4.5%)	0.658
Increased ALT	11 (22.9%)	11 (25.0%)	0.815	1 (2.1%)	1 (2.3%)	0.731
Thrombocytopenia	7 (14.6%)	7 (15.9%)	0.860	0	0	/
Dysphonia	5 (10.4%)	8 (18.2%)	0.285	1 (2.1%)	2 (4.5%)	0.467
Hypothyroidism	6 (12.5%)	6 (13.6%)	0.872	0	0	/

ALT, alanine transaminase.

lenvatinib can suppress the development of liver fibrosis in preclinical experiments (59) and help maintain a liver functional reserve in the patients (60). Since the present study suggests that lenvatinib plus camrelizumab has promising efficacy in patients with intermediate-stage HCC, it will be worth exploring whether combining lenvatinib and camrelizumab with TACE or HAIC might have additional clinical benefits.

The use of camrelizumab monotherapy can lead to reactive cutaneous capillary endothelial proliferation (RCCEP), but the incidence of this adverse effect is decreased significantly if camrelizumab is combined with a targeted anti-angiogenic drug. Therefore, none of the patients in this study were treated with camrelizumab alone, and as a result, there were no cases of RCCEP. The multivariable regression analysis showed that, in addition to combination therapy, the ECOG PS score was associated with prognostic outcomes (OS and PFS). The ECOG PS score is a well-known prognostic factor in patients with cancer (61, 62). In addition, our findings showed that hypertension and proteinuria were associated with a longer OS, suggesting that the occurrence of hypertension and proteinuria might be indicators of good treatment response. Similar results were observed with bevacizumab in patients with glioblastoma (63) and antiangiogenic therapies in metastatic colorectal cancer (64). In HCC treated with sorafenib, the occurrence of off-target AEs including hypertension, diarrhea, skin toxicity, and fatigue have been shown to be positively related to better treatment response of time to progression and OS (65). Furthermore, some immune-related AEs with anti-PD-1 therapies have been associated with a good prognosis in patients with colorectal cancer and non-small cell lung cancer (66–68), but this association has not been previously reported for camrelizumab in patients HCC. Therefore, patients with such AEs should be managed appropriately and should be encouraged to continue treatment since these AEs might be predictive of treatment response. It will require further investigation in future studies.

TABLE 4 | Multivariable Cox regression analysis for OS.

	Univariable analysis			Multivariable analysis		
	HR	95% CI	P	HR	95% CI	P
Combined therapy vs. monotherapy	0.477	0.259-0.881	0.018	0.380	0.196-0.739	0.004
Body mass index	0.917	0.823-1.021	0.112			
ECOG PS			<0.001			0.003
1 vs. 0	1.842	0.907-3.741	0.091	1.298	0.610-2.764	0.498
2 vs. 0	5.631	2.375-13.35	<0.001	6.769	2.183-20.989	0.001
Child-Pugh level (C vs. B)	2.496	1.363-4.571	0.003	1.405	0.516-3.821	0.506
AFP level (>200 vs. ≤200)	1.063	0.584-1.934	0.841			
Tumor number (>3 vs. ≤3)	1.441	0.641-3.241	0.377			
BCLC stage (C vs. B)	2.887	0.890-9.362	0.077	1.749	0.462-6.614	0.410
HBV infection	2.836	0.887-9.173	0.082	2.321	0.637-8.46	0.202
Vascular invasion (yes vs. no)	1.727	0.799-3.733	0.165			
Intrahepatic metastasis (yes vs. no)	1.521	0.705-3.281	0.285			
Extrahepatic metastasis (yes vs. no)	2.03	1.111-3.710	0.021	1.568	0.795-3.09	0.194
Hand-foot syndrome (yes vs. no)	0.932	0.459-1.893	0.847			
Hypertension (yes vs. no)	0.272	0.121-0.613	0.002	0.393	0.163-0.944	0.037
Proteinuria (yes vs. no)	0.11	0.034-0.355	<0.001	0.196	0.054-0.704	0.012
Dysphonia (yes vs. no)	2.5	1.221-5.119	0.012	2.386	1.022-5.57	0.044

HR, hazard ratio; CI, confidence interval; ECOG PS, Eastern Cooperative Oncology Group performance status; AFP, α-fetoprotein; BCLC, Barcelona Clinic Liver Cancer; HBV, hepatitis B virus.

TABLE 5 | Multivariable Cox regression analysis for PFS.

	Univariable analysis			Multivariable analysis		
	HR	95% CI	P	HR	95% CI	P
Combined therapy vs. monotherapy	0.567	0.363-0.885	0.012	0.454	0.282-0.731	0.001
Body mass index	0.954	0.883-1.031	0.23			
ECOG PS			<0.001			
1 vs. 0	1.104	0.684-1.781	0.685	0.940	0.571-1.546	0.807
2 vs. 0	2.773	1.436-5.358	0.002	2.955	1.416-6.166	0.004
Child-Pugh level (C vs. B)	2.242	1.143-4.399	0.019	1.584	0.742-3.385	0.235
AFP level (>200 vs. ≤200)	1.019	0.659-1.574	0.934			
Tumor number (>3 vs. ≤3)	1.724	0.941-3.161	0.078			
BCLC stage (C vs. B)	1.097	0.592-2.035	0.768			
HBV infection	2.106	1.048-4.232	0.036	1.813	0.828-3.968	0.136
Vascular invasion (yes vs. no)	1.027	0.619-1.704	0.919			
Intrahepatic metastasis (yes vs. no)	1.869	1.058-3.300	0.031	1.494	0.820-2.723	0.190
Extrahepatic metastasis (yes vs. no)	1.347	0.869-2.088	0.183	1.568	0.795-3.09	0.194
Hand-foot syndrome (yes vs. no)	1.251	0.758-2.067	0.381			
Hypertension (yes vs. no)	0.868	0.551-1.368	0.542			
Proteinuria (yes vs. no)	0.557	0.340-0.913	0.020	0.598	0.335-1.067	0.082
Dysphonia (yes vs. no)	1.702	0.947-3.060	0.075	1.500	0.804-2.798	0.202

HR, hazard ratio; CI, confidence interval; ECOG PS, Eastern Cooperative Oncology Group performance status; AFP, α-fetoprotein; BCLC, Barcelona Clinic Liver Cancer; HBV, hepatitis B virus.

The combination of bevacizumab and atezolizumab is expensive, with healthcare costs of $313,193 compared to $156,984 for sorafenib and an incremental cost-effectiveness ratio of $322,500 per quality-adjusted life-year (69). Therefore, many healthcare insurances do not reimburse the costs of this combination, and many patients cannot afford or have access to such a regimen. In China, lenvatinib is already covered by medical insurance as first-line therapy for HCC, and thus the accessibility of lenvatinib plus camrelizumab is higher. Additionally, camrelizumab has a remarkable price advantage in China (2928 RMB/cycle or USD 2300 a year), where it was developed and has been widely applied for cancer therapy, especially since it is covered by national medical insurance.

The subgroup analyses suggested that patients with specific characteristics might benefit more than others from the lenvatinib plus camrelizumab combination. However, the results of the subgroup analyses must be interpreted with caution because some subgroups were small and had few events. The study was not powered to reach firm conclusions about these subgroup analyses. Nevertheless, males, patients with Child-Pugh score ≤7, >3 tumors, AFP level >200 mg/dL, HBV infection or vascular invasion, and patients without hypertension might benefit more than their counterparts. Additional studies are needed to verify which patients might exhibit better outcomes after treatment with lenvatinib and camrelizumab. Moreover, large-scale studies comparing the therapeutic and adverse effects of different combinations of drugs in different patient subgroups might in the future allow for individualized therapies to be selected based on the clinical characteristics of the patient.

This study has limitations. It was a retrospective study with a relatively small sample size. The analyzable data were limited to those available in the medical charts. Furthermore, the follow-up was relatively short, and the data for several endpoints, such as OS, were still immature. Additional studies and randomized controlled trials should be performed to confirm these results. Such a trial (ClinicalTrials.gov NCT04443309) is currently underway.

In conclusion, first-line therapy with lenvatinib plus camrelizumab might benefit patients with unresectable HCC more than lenvatinib monotherapy. The toxicity profile and tolerability appeared similar between the two therapeutic regimens, and there were no new safety signals. Combined therapy with lenvatinib and camrelizumab might provide a new treatment option for patients with unresectable HCC and is worth further investigating.

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 The Ethics Committees of all four study centers. The requirement for individual informed consent was waived by the committees.

AUTHOR CONTRIBUTIONS

QL, MRC, and GY have full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the

data analysis. JC and JR were involved in the study conceptualization and design. All authors (QL, MRC, GY, XC, MZ, MC, XH, JH, YG, RL, JR, and JC) were involved in the acquisition, analysis, and interpretation of data. QL and JR supervised the analysis. QL and JC were involved in the drafting of the manuscript. All authors read, critically revised, and approved the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2022.809709/full#supplementary-material

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Effects of Stereotactic Body Radiation Therapy Plus PD-1 Inhibitors for Patients With Transarterial Chemoembolization Refractory

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Background and Aims: Patients with intermediate-stage hepatocellular carcinoma (HCC) who are refractory to transarterial chemoembolization (TACE) have a poor prognosis. This study aimed to explore whether stereotactic body radiation therapy (SBRT) combined with PD-1 inhibitors could improve the clinical outcomes of such patients.

Methods: This retrospective cohort study included patients with intermediate-stage HCC who were diagnosed with TACE refractoriness between January 2019 and December 2020 in the Eastern Hepatobiliary Surgery Hospital and the First Affiliated Hospital of Wenzhou Medical University. The patients were divided into two groups: (1) those who switched from TACE to receive stereotactic body radiotherapy (SBRT) combined with PD-1 inhibitors; (2) those who continued TACE treatment and added PD-1 inhibitors. Progression-free survival (PFS), overall survival (OS), and tumour response were assessed in both groups after becoming refractory to TACE treatment.

Results: Of the seventy-six patients included in this study, the median PFS was 19.6 months in the SBRT-IO group (n=31) and 10.1 months in the TACE-IO group (n=45, p<0.05). The SBRT-IO group also had a significantly higher OS than the TACE-IO group (p<0.05). The objective response rate (ORR) and disease control rate (DCR) were also better in the SBRT-IO group (ORR, 71.0% vs. 15.6%, OR=8.483, 95% CI 3.319-21.680, P < 0.001; DCR, 80.6% vs. 31.1%, OR=9.226, 95% CI 3.096-27.493, P < 0.001).

Conclusions: SBRT combined with a PD-1 inhibitor improves PFS and OS in TACE-refractory patients with intermediate-stage HCC. Therefore, this therapy is a suitable option in cases of TACE treatment failure.

Keywords: hepatocellular carcinoma, stereotactic body radiation therapy, transarterial chemoembolization refractory, immunotherapy, combination therapy

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INTRODUCTION

Hepatocellular carcinoma (HCC) is the most common primary liver cancer and the fourth leading cause of cancer-related death worldwide (1). Since patients with early-stage HCC are usually asymptomatic, approximately half of them are diagnosed at intermediate to advanced stages and cannot undergo radical treatment (2–5).

For patients with intermediate-stage HCC, transarterial chemoembolization (TACE) is recommended as the standard treatment by many guidelines (6–9). However, the efficacy of TACE alone is limited, and some patients are diagnosed as refractory to TACE (10, 11). Most guidelines recommend starting systemic therapy as soon as TACE refractoriness occurs (6, 8, 9). As a new systemic therapeutic drug, PD-1 inhibitors show synergistic effects when combined with TACE (12, 13). In other words, the combined use of PD-1 inhibitors may improve the prognosis of TACE-refractory patients.

Stereotactic body radiotherapy (SBRT) is a newer treatment with evidence of promising local control for patients with HCC (14–16). For early- and intermediate-stage HCC patients, SBRT is a safe alternative to TACE and provides no inferior or even better local control and overall survival (OS) than TACE (17, 18). Furthermore, there is synergy in the use of radiotherapy in combination with PD-1 inhibitors (19, 20). Therefore, we speculate that SBRT combined with a PD-1 inhibitor may be an effective alternative treatment for TACE-refractory patients.

In this study, we investigated whether TACE-refractory patients should be administered PD-1 inhibitors to maintain TACE treatment or should be switched to SBRT plus PD-1 inhibitors, as reports on these two treatments are currently lacking. We conducted this retrospective study to evaluate the efficacy and safety of the above two therapies in intermediate HCC patients who are refractory to TACE treatment.

METHODS

Patients

A retrospective study of consecutive HCC patients was conducted at the Eastern Hepatobiliary Surgery Hospital and the First Affiliated Hospital of Wenzhou Medical University from 2019 to 2020. This study was approved by the Institutional Ethics Committee of each centre. As patient identities were anonymized, the requirement for informed consent was waived by the ethics committee.

The inclusion criteria were patients with (1) HCC diagnosed by histopathology, computed tomography (CT) or magnetic resonance imaging (MRI), (2) good liver function (Child-Pugh A or B7, score <= 7), (3) BCLC stage B, (4) TACE, and (5) TACE refractoriness. The exclusion criteria were patients with (1) previous locoregional or systemic therapy, (2) recurrent HCC, (3) a history of other cancers, and (4) incomplete clinical data.

TACE and SBRT

The optimal treatment modality was discussed and determined by the multidisciplinary team at each institution. Locoregional therapies, including surgery or alternative approaches (SBRT or TACE), are considered based on the individual patient's circumstances (tumour size, liver function, and proximity to organs at risk). The final decision is made by the patient after the benefits of various treatment modalities, as well as associated side effects and costs, have been fully explained.

TACE was performed as previously described using the Seldinger's technique (21). Briefly, the tumour-feeding artery was first identified by angiography, and after cannulation of the hepatic artery, doxorubicin hydrochloride, pirarubicin and lipiodol were injected through the catheter. Post TACE evaluation and follow-up were performed every 6-8 weeks. The diagnostic criteria of TACE refractoriness were based on the definition proposed by the Japan Society of Hepatology (JSH) and the Liver Cancer Study Group of Japan (insufficient response of the treated tumour after two procedures) (22).

SBRT was performed by CyberKnife® (Accuray Cyberknife, VSI), with a total of 24–45 Gy in 3–5 fractions. The patients who received SBRT were first implanted with at least 3 gold fiducials inside or adjacent to the tumour under CT (Philips Brilliance CT Big Bore Oncology) guidance, and the gold fiducials were relatively stable and immobile after seven days, with localization simulated under CT. The images were subsequently transferred to the treatment planning system, and the target area was then delineated by a radiologist. A 2-5 mm marginal expansion of the gross tumour volume (defined as radiologically evident gross disease) formed the planning target volume. The physiatrist developed the treatment plan while defining normal tissue dose ranges. Dose-volume histograms were generated for all target volumes and critical normal structures. Dose constraints for organs at risk were determined based on the American Association of Physicists in Medicine guidelines in AAPM Task Group 101 (23).

PD-1 Inhibitors

All included patients were treated with PD-1 inhibitors after being diagnosed as refractory to TACE treatment. PD-1 inhibitors included toripalimab (72.4%) and sintilimab (27.6%) (**Supplementary Table 1**), both of which have been reported to be effective in patients with HCC (24–31). Toripalimab was administered at a dose of 3 mg/kg by body weight every 2 weeks; sintilimab was administered at a dose of 200 mg every three weeks. The specific doses and protocols used were strictly in accordance with the instructions for use. PD-1 inhibitors were all administered intravenously; if low-grade infusion reactions occurred, drip plasticity was reduced or dosing was suspended until the symptoms resolved, at which time the medication was resumed while the patient remained under close observation. PD-1 inhibitors were continued until intolerable toxicity occurred.

Abbreviations: HCC, hepatocellular carcinoma; TACE, transarterial chemoembolization; SBRT, stereotactic body radiation therapy; OS, overall survival; CT, computed tomography; MRI, magnetic resonance imaging; JSH, Japan Society of Hepatology; PFS, progression-free survival; TRAEs, treatment-related adverse events; HR, hazard ratio; OR, odds ratio; ORR, objective response rate; DCR, disease control rate; DAMPs, damage-associated molecular patterns.

Follow–Up and Assessment

All patients visited the outpatient clinic for follow-up every 1-3 months. At each follow-up visit, a routine physical examination, laboratory blood tests, and abdominal ultrasound or enhanced CT/MRI were performed. The primary outcome of this study was progression-free survival (PFS), which was defined as the time from the initiation of PD-1 inhibitors to tumour progression, death from any cause, or the most recent follow-up. The secondary endpoints included overall survival (OS), objective response rate (ORR) and treatment-related adverse events (TRAEs). Tumour progression included progression of treated lesion, and new lesions within or outside the liver. OS was defined as the time from the initiation of PD-1 inhibitor use until the date of death from any cause or the date of the most recent follow-up visit. Disease control rate (DCR) was defined as percentage of patient attained complete response, partial response or stable disease. Assessment of tumour progression was based on modified Response Evaluation Criteria in Solid Tumours criteria (mRECIST).

TRAEs were recorded from the initiation of PD-1 inhibitor use and obtained from clinical visit notes or medical records. TRAEs were assessed according to the criteria of the common terminology criteria for adverse events (CTCAE, version 5.0). If multiple instances of the same type of toxicity occurred, the highest grade for each patient in a given category was adopted.

Statistical Analysis

All clinical data were analysed using IBM SPSS Statistics 24 (New York, NY, USA) or R 4.0 software (http://www.r-project.org/). Student's t-test was used to compare continuous variables, and the $\chi 2$ test or Fisher exact test was used to compare categorical variables. Survival curves were calculated using the Kaplan–Meier method and compared using the log-rank test. The hazard ratio (HR) was calculated by Cox regression models. Univariate Cox regression analysis was used to evaluate the significance of variable in the entire cohort. All variables which were significantly related to PFS ($p<0.05$) were included in the multiple Cox regression analysis. The odds ratio (OR) was calculated by logistic regression models. $P < 0.05$ was considered to indicate a significant difference.

RESULTS

Patient Characteristics and Treatments

A flow diagram of the present study is shown in **Figure 1**. Of the 76 patients at the Eastern Hepatobiliary Surgery Hospital and the First Affiliated Hospital of Wenzhou Medical University with complete clinical and follow-up data, 45 (59.2%) patients received TACE-IO therapy, and 31 (47.3%) received SBRT-IO therapy. **Table 1** summarizes the baseline features of these patients. There were no significant differences at baseline between the two groups, including age, sex, HBsAg, maximum tumour size, number of tumours, alpha-fetoprotein concentration, Des-gamma-carboxy prothrombin, total bilirubin, albumin, albumin-bilirubin grade, prothrombin time, glucose, creatinine or platelet count.

The median follow-up was 10 and 11 months in the TACE-IO and SBRT-IO groups, respectively. The median cycle of PD-1 inhibitor use was six in both groups. Total 63 lesions were irradiated in SBRT-IO arm (Single lesion, n=5; Two lesions, n=20; Three lesions, n=6). Of the 76 patients enrolled in the study, 41 patients died during the study (31 in the TACE-IO group and 10 in the SBRT-IO group), 29 were alive (10 in the TACE-IO group and 19 in the SBRT-IO group), and 6 were lost to follow-up (4 in the TACE-IO group and 2 in the SBRT-IO group).

Efficacy Outcomes

The median PFS was 19.6 months (95% CI 13.1-26.1) in the SBRT-IO group and 10.1 months (95% CI 7.3-12.9) in the TACE-IO group. The median OS was 14.1 months in the TACE-IO

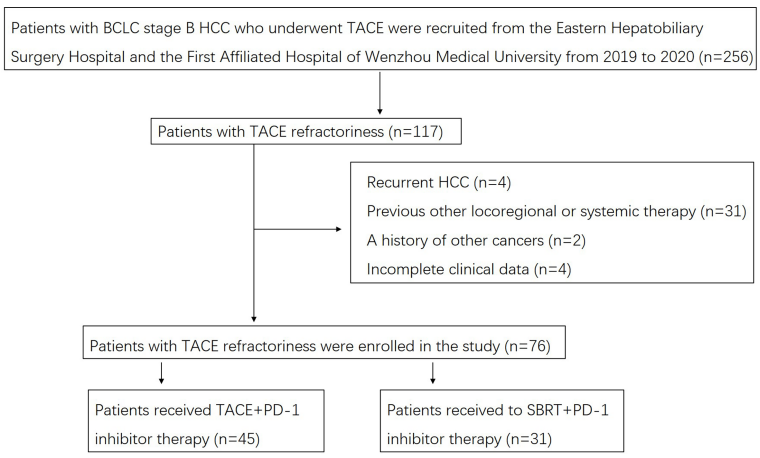

FIGURE 1 | Flow diagram for the present study. BCLC, Barcelona Clinic Liver Cancer; HCC, hepatocellular carcinoma; TACE, transarterial chemoembolization; SBRT, stereotactic body radiation therapy.

TABLE 1 | Baseline characteristics of study patients.

Characteristics	TACE-IO (n = 45)	SBRT-IO (n = 31)	*P* value
Age, year			0.945
< 65	36 (86.2)	25 (78.8)	
≥ 65	9 (13.8)	6 (21.2)	
Gender			0.168
Female	6 (13.8)	8 (19.2)	
Male	39 (86.2)	23 (80.8)	
HBsAg			0.525
Positive	40 (82.8)	26 (78.9)	
Negative	5 (17.2)	5 (21.1)	
Liver cirrhosis			0.666
Yes	38 (84.4)	25 (80.6)	
No	7 (15.6)	6 (19.4)	
Child-Pugh			1.000
A	44	31	
B7	1	0	
Maximum tumour size, cm, median (range)	4.8 (1.3-12)	4.3 (1.6-6)	0.161
Tumour number			0.137
2	31	20	
3	10	11	
4	4	0	
AFP, ng/mL			0.610
< 400	22 (58.6)	17 (61.5)	
≥ 400	23 (41.4)	14 (38.5)	
DCP, mAU/mL			0.555
< 2050	26 (65.5)	20 (73.1)	
≥ 2050	19 (34.5)	11 (26.9)	
TB, umol/L			0.468
< 18.8	27	16	
≥ 18.1	18	15	
Albumin, g/L			0.145
< 35	8	10	
≥ 35	37	21	
ALBI grade			0.243
1	25 (55.2)	13 (53.9)	
2	20 (41.4)	18 (46.1)	
3	0	0	
PT, sec			0.669
< 13	36	26	
≥ 13	9	5	
Glucose, mmol/L			0.337
< 7	39	29	
≥ 7	6	2	
Creatinine, umol/L, median	66.0	61.0	0.222
Platelet, $X10^9$, median	162.0	174.0	0.625

TACE, transcatheter arterial chemoembolization; SBRT, stereotactic body radiation therapy; AFP, alpha-fetoprotein concentration; DCP, Des-gamma-carboxy prothrombin; TB, total bilirubin; ALBI, albumin-bilirubin; PT, prothrombin time.

group and was not reached in the SBRT-IO group. The 1-year OS and PFS rates of the SBRT-IO group were 71.5% and 64.8%, respectively, while those of the TACE-IO group were 54.2% and 40.7%, respectively. SBRT significantly prolonged PFS relative to TACE (**Figure 2A**, P < 0.05). In the entire cohort, treatment with SBRT-IO was a significantly unfavourable factor for PFS (HR=0.372, 95% CI 0.186-0.745, P=0.005), along with ALBI grade 2 (**Table 2**). Similarly, as shown in **Figure 2B**, SBRT significantly prolonged OS relative to TACE (HR = 0.375, 95% CI 0.182-0.773, P < 0.05).

Table 3 summarizes the best tumour responses for all HCC patients. According to mRECIST, the ORR in the SBRT-IO group was significantly higher than that in the TACE-IO group (71.0% vs. 15.6%, OR=8.483, 95% CI 3.319-21.680, P < 0.001). The DCR in the SBRT-IO group was also significantly higher than that in the TACE-IO group (80.6% vs. 31.1%, OR=9.226, 95% CI 3.096-27.493, P < 0.001).

First Site of Progressive Disease and Treatment on Progression

Forty-five patients had progressed at the time of analysis. Thirty-one (68.9%) patients in the TACE-IO cohort progressed, 26 (57.8%) of whom had intrahepatic progression; Fourteen (45.2%) patients progressed in the SBRT-IO cohort, 11 (35.5%) of whom had intrahepatic progression, as detailed in **Supplementary Table 2**.

Following progressive disease, most patients had more treatment. In the TACE-IO cohort, 27 patients received further treatment, three patients received supportive care due to physical

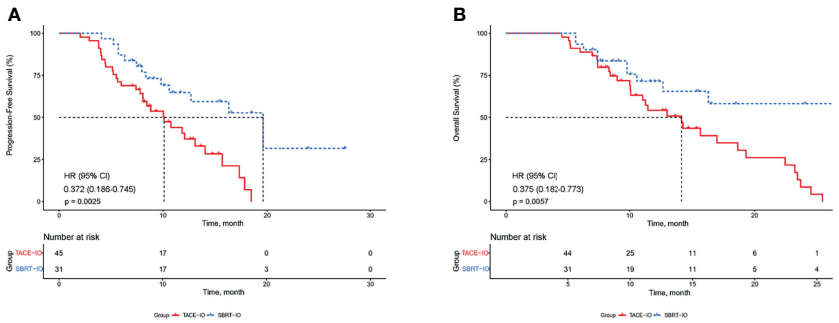

FIGURE 2 | Kaplan–Meier estimated PFS and OS curves of HCC patients receiving different therapies. **(A)** PFS; **(B)** OS. PFS, progression-free survival; OS, overall survival; HCC, hepatocellular carcinoma; TACE, transarterial chemoembolization; SBRT, stereotactic body radiation therapy.

deterioration, and one patient refused treatment; In the SBRT-IO cohort, 12 patients received further treatment, one patient received supportive care and one patient refused treatment.

Safety Outcomes

According to CTCAE version 5.0, TRAEs were evaluated during treatment according to their frequency and severity. Almost all patients experienced transient TRAEs after receiving locoregional therapies, which spontaneously resolved. Therefore, we did not analyse and discuss these transient TRAEs.

As shown in **Supplementary Table 3**, the most common TRAEs at all levels in the TACE-IO group were decreased platelet count (44.4%), decreased albumin (37.8%), and elevated AST (37.8%). In addition, the most common grade 3/4 TRAE was decreased platelet count (6.7%). In the SBRT-IO group, the most common TRAEs were fatigue (54.8%), decreased platelet count (48.4%) and decreased white blood cell (32.3%), and the most common grade 3/4 TRAEs were elevated AST (3.2%) and ALT (3.2%) levels, and hand-foot skin reaction (3.2%). Furthermore, among patients treated with SBRT-IO, none developed classical radiation-induced liver disease, and no treatment-related deaths occurred.

DISCUSSION

In this study, we report for the first time the efficacy of SBRT combined with a PD-1 inhibitor in TACE-refractory patients with intermediate-stage HCC. The results showed that receiving SBRT combined with a PD-1 inhibitor provided a better long-term prognosis and greater tumour control than TACE combined with a PD-1 inhibitor alone for TACE refractory patients. This provides more options for the treatment of patients with BCLC stage B HCC.

TACE is the standard of care for patients with BCLC stage B HCC (6–9), but some patients develop TACE refractoriness and cannot achieve effective tumour control (10, 11). The guidelines recommend that patients start receiving systemic therapy once

TABLE 2 | Prognostic factors for progression-free survival.

Progression-free survival	Univariate analysis			Multivariate analysis		
	HR	95% CI	P value	HR	95% CI	P value
Age (>65/≤65 years)	1.459	0.669-3.183	0.342			
Sex (male/female)	2.029	0.857-4.808	0.108			
HBsAg (positive/negative)	0.614	0.283-1.334	0.218			
Albumin (>35/≤35 g/dl)	0.638	0.296-1.373	0.250			
Total bilirubin (>17.1/≤17.1 μmol/L)	1.524	0.838-2.773	0.167			
ALBI grade (2/1)	2.234	1.200-4.159	0.011	2.132	1.144-3.970	**0.017**
Prothrombin time (>13/≤13 second)	0.866	0.361-2.076	0.747			
Creatinine (>106/≤106 μmol/L)	1.001	0.982-1.021	0.896			
Blood glucose (>7/≤7 mmol/L)	1.031	0.401-2.649	0.950			
Platelet (>100/≤100 10^9/L)	0.995	0.988-1.003	0.216			
Alpha fetoprotein (≥400/<400 ng/mL)	0.770	0.427-1.391	0.386			
DCP (≥2050/<2050 mAU/mL)	0.730	0.396-1.346	0.314			
Liver cirrhosis (yes/no)	1.080	0.517-2.255	0.838			
Tumour number (3/2)	1.044	0.533-2.042	0.901			
Tumour number (4/2)	3.022	0.901-10.141	0.073			
Maximum tumour size, cm	1.126	0.575-2.205	0.730			
Treatment (SBRT-IO/TACE-IO)	0.361	0.182-0.716	0.004	0.372	0.186-0.745	**0.005**

ALBI, albumin-bilirubin; DCP, Des-gamma-carboxy prothrombin; SBRT, stereotactic body radiation therapy; TACE, transcatheter arterial chemoembolization.

TABLE 3 | Best tumour response.

	TACE-IO (n=45)	SBRT-IO (n=31)	*P* value
CR	0	11(35.5)	
PR	7 (15.6)	11 (35.5)	
SD	7 (15.6)	3 (9.7)	
PD	31 (68.9)	6 (19.4)	
ORR	7 (15.6)	22 (71.0)	<0.001
DCR	14 (31.1)	25 (80.6)	<0.001

TACE, transcatheter arterial chemoembolization; SBRT, stereotactic body radiation therapy; CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease; ORR, objective response rate; DCR, disease control rate.

they are diagnosed with TACE refractoriness (6, 8, 9). However, a recent international expert panel of International Society of Multidisciplinary Interventional Oncology consensus statement and a survey by Chinese College of Interventionalists indicated that repeated TACE especially TACE based combination therapy can also achieve survival benefit in patients refractory to TACE (32–35). Meanwhile, PD-1 inhibitors have been increasingly explored as representative agents for immunotherapy, the possible mechanism underlying the benefit of TACE combined with a PD-1 inhibitor was revealed: TACE could decrease the ratio of CD4+/CD8+ cells and increase the level of PD-1 mRNA expression in patients with HCC (12). Therefore, TACE combined with a PD-1 inhibitor might have potential clinical value for patients who are refractory to TACE.

Radiotherapy is limited in its clinical application in these patients because of increased hepatotoxicity. Due to technological advances, SBRT is currently able to safely deliver high-dose radiotherapy to HCC patients, and the American Association for the Study of Liver Diseases guidelines accept SBRT as one of the treatments for HCC (7). A previous study showed that patients with intermediate- and advanced-stage HCC can also benefit from SBRT (36), and another study demonstrated that the 2-year local control rate reached 61-81% in patients with BCLC stage B HCC who received SBRT (37). Several retrospective controlled studies involving patients with intermediate-stage HCC showed that SBRT had similar or even higher tumour control rates and OS rates than TACE (17, 18), and one clinical trial demonstrated the safety and feasibility of SBRT as a local salvage regimen for patients with an incomplete response to TACE (38). On the one hand, the benefit of SBRT for patients with HCC is guaranteed, while on the other hand, the potential benefit of combining SBRT with a PD-1 inhibitor has been revealed. In terms of the underlying mechanism, radiotherapy can trigger immunogenic cell death, resulting in the release of cytokines and damage-associated molecular patterns (DAMPs). DAMPs can lead to the subsequent priming and trafficking of tumour-specific T lymphocytes into the tumour microenvironment by enhancing the recruitment of antigen-presenting cells, the processing of tumour-associated antigens, and the cross presentation of antigenic peptides on major histocompatibility complex class I, thereby enhancing the efficacy of PD-1 inhibitors (20). Its clinical benefits have also been reported (38–40).

Based on the above findings, we speculate that intermediate-stage HCC patients who are refractory to TACE might benefit from the addition of a PD-1 inhibitor or from the switch to SBRT combined with a PD-1 inhibitor. In this study, which enrolled 76 patients proven to be refractory to PD-1 inhibitor TACE treatment, the SBRT-IO group (n = 31) had a median PFS of 19.6 months (95% CI 13.1-26.1), which was significantly higher than the TACE-IO group (n = 45) with a median PFS of 10.1 months (95% CI 7.3-12.9, P < 0.001). The 1-year OS and 1-year PFS rates in the SBRT-IO group were 71.5% and 64.8%, and the ORR and DCR were 71.0% and 80.6%, respectively. The 1-year OS and 1-year PFS rates in the TACE-IO group were 54.2% and 40.7%, and the ORR and DCR were 15.6% and 31.1%, respectively. Compared with TACE-IO, SBRT-OI significantly prolonged PFS (HR=0.372, 95% CI 0.186-0.745, P=0.005) and OS (HR = 0.375, 95% CI 0.182-0.773, P < 0.001) and resulted in a better ORR (OR=8.483, 95% CI 3.319-21.680, P < 0.001) and DCR (OR = 9.226, 95% CI 3.096-27.493, P < 0.001) in TACE-refractory patients. Furthermore, the median OS of the TACE-IO group was similar to that of TACE-refractory patients as previously reported by Kudo et al. (41); therefore, whether adding a PD-1 inhibitor can improve the prognosis for TACE-refractory patients requires further study.

In addition to efficacy, we analysed the TRAEs associated with SBRT plus PD-1 inhibitors. The most common TRAEs were decreased WBC (67.7%), fatigue (54.8%) and decreased platelet count (48.4%), and the most common grade 3/4 TRAEs were decreased WBC (6.5%), elevated AST (3.2%) and ALT (3.2%) levels and hand-foot skin reaction (3.2%), with no unexpected TRAEs occurring. Therefore, SBRT-IO is an effective and safe treatment for intermediate-stage HCC patients who are refractory to TACE treatment.

We must acknowledge that our study had some limitations. First, this is a retrospective study with inherent defects. Second, this was a study conducted in HBV-endemic China, which may have influenced the results. Third, the sample size included in this study was small, and the number of tumours per patient was small (fewer than 5). A prospective study is therefore needed to confirm our findings.

In conclusion, our data strongly support the fact that switching to a combination of SBRT and a PD-1 inhibitor improves clinical outcomes, as evidenced by the increased PFS and OS in intermediate-stage HCC patients who are refractory to TACE. Repeated TACE treatments may cause resistance to systemic therapy and result in the deterioration of liver function. Therefore, the combination of SBRT with a PD-1 inhibitor is a safe and effective alternative that warrants consideration by clinicians.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

This study was approved by the Institutional Ethics Committees of the Eastern Hepatobiliary Surgery Hospital and the First Affiliated Hospital of Wenzhou Medical University. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHORS CONTRIBUTIONS

Conceptualization: S-QC, Y-FS, and Y-JX. Funding acquisition: S-QC, KW. Resources: S-QC, Y-FS, KW, SF, XC, H-MY, X-WL, L-PZ, JZ, YM. Investigation: Y-JX, KW, Y-TZ, SF, H-MY, Y-QC. Formal analysis: Y-JX, KW, Y-TZ, SF. Writing–original draft: Y-JX. Writing–review & editing: S-QC and Y-FS. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2022.839605/full#supplementary-material

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ORIGINAL RESEARCH
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Application Effect of ICG Fluorescence Real-Time Imaging Technology in Laparoscopic Hepatectomy

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This study aimed to evaluate the efficiency and safety of indocyanine green (ICG) fluorescence real-time imaging-guided technology in laparoscopic hepatectomy. A retrospective analysis of patients with primary liver cancer in the First Affiliated Hospital of USTC from January 2018 to October 2021, including 48 cases of fluorescence-guided laparoscopic hepatectomy (FGLH) and 60 cases of traditional laparoscopic hepatectomy (LH), was conducted. R0 resection rate, operation time, intraoperative blood loss, complications, hospital stay, and other intraoperative and postoperative indicators of the two groups were analyzed to determine the clinical feasibility and safety of ICG fluorescence real-time imaging-guided technology in laparoscopic hepatectomy. Related databases were searched for retrospective cohort studies and randomized controlled trials comparing FGLH with LH, studies were screened according to preset inclusion and exclusion criteria, literature quality was evaluated, and data were extracted. RevMan 5.3 software was used to conduct a meta-analysis on the extracted data. The results of our clinical data and meta-analysis showed that compared with LH, FGLH increased the R0 resection rate, shortened the operation time and postoperative hospital stay, and reduced blood loss and the occurrence of postoperative complications. Compared with LH, FGLH has a better application effect in laparoscopic hepatectomy, and it is worthy of promotion as it is safe and feasible.

Keywords: laparoscopic hepatectomy, indocyanine green, fluorescence imaging, meta-analysis, hepatocellular carcinoma (HCC)

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1 INTRODUCTION

Primary liver cancer is one of the most common malignant tumors of the digestive system. Globally, it ranks sixth in incidence and second in mortality (1). Hepatocellular carcinoma has the characteristics of metastasis along the tumor-bearing portal vein system. Anatomical hepatectomy removes the main tumor and also completely removes the micrometastasis in the tumor-bearing liver segment (2). Even if the resection margin is large enough, there may be residual metastases in the portal vein system of the tumor-bearing liver segment, resulting in early recurrence of non-anatomical hepatectomy (3, 4). Laparoscopic hepatectomy has been widely used in patients with liver tumors, but it is difficult to implement the laparoscopic anatomical hepatectomy procedures advocated by Makuuchi et al. (5) and visually determine the boundaries of liver segments on the monitor. Therefore, the accuracy of laparoscopic anatomical hepatectomy will be affected, but laparoscopic indocyanine green staining of the liver segment can make up for this deficiency (6–8). At present, the safety and effectiveness of indocyanine green (ICG) imaging technology in laparoscopic hepatectomy are still controversial (9, 10). This study retrospectively analyzed the clinical data of 108 patients undergoing laparoscopic hepatectomy from January 2018 to October 2021 in the Department of Hepatic Surgery of the First Affiliated Hospital of the University of Science and Technology of China, integrated relevant clinical studies in recent years to conduct a meta-analysis, and then discussed the safety and application effect of ICG fluorescence real-time imaging-guided technology in laparoscopic hepatectomy.

2 MATERIALS AND METHODS

2.1 Clinical Data

2.1.1 General Information

A total of 108 patients who met the inclusion criteria and underwent laparoscopic hepatectomy were recruited from January 2018 to October 2021 in the First Affiliated Hospital of USTC (University of Science and Technology of China). The patients were divided into the experimental group [fluorescence-guided laparoscopic hepatectomy (FGLH)] with 48 cases and the control group [traditional laparoscopic hepatectomy (LH)] with 60 cases. All patients had postoperative pathologically confirmed primary liver cancer, and there was no statistically significant difference in preoperative baseline data between the two groups (**Table 1**). All patients signed an informed consent form before surgery, which complied with medical ethics requirements.

2.1.2 Inclusion Criteria and Exclusion Criteria

According to the indications and contraindications of laparoscopic hepatectomy and ICG fluorescence-guided technology reported in the literature, the following inclusion criteria of laparoscopic hepatectomy were formulated: a) the patient is generally in good condition and important organs do not have serious dysfunction, such as the heart, brain, and lung; b) good liver reserve function; c) no important vascular invasion and venous tumor thrombus; and d) no distant tumor metastasis.

The exclusion criteria were as follows: a) the general condition of the patient is poor and cannot tolerate surgery or long-term pneumoperitoneum, b) allergic to iodine or ICG, c) moderately or severely impaired liver reserve function, d) large tumor and unable to undergo complete laparoscopy resection, e) the tumor is close to the hilum of the liver or invades large blood vessels, f) preoperative imaging shows multiple metastases in the liver or distant metastases, and g) postoperative pathologically confirmed non-liver cancer.

2.1.3 Surgical Methods

Laparoscopic hepatectomy uses the traditional five-hole method. After exposing the Glisson system of the liver segment where the tumor is located, the target liver segment is stained. There are two types of staining methods. a) Positive staining: Under the guidance of percutaneous or laparoscopic ultrasound, the corresponding liver segment or subsegment portal vein is located or the corresponding liver segment or subsegment Glisson is isolated, indocyanine green is injected into the corresponding liver segment or subsegment portal vein (0.25~0.5 mg), and then the stained liver is completely removed (**Figure 1** and **Video S1**). b) Negative staining: After isolating the corresponding liver segment or subsegment Glisson, the corresponding Glisson system is disconnected, indocyanine green (0.75~1.25 mg) is injected through the peripheral vein, and the unstained part of the liver is completely removed. If the portal vein branch of the target liver segment is thin, or the Glisson pedicle of the target liver segment is difficult to expose and the staining fails, intraoperative Doppler ultrasound will be used to define the hepatectomy margin at a distance of 1–2 cm from the tumor boundary. In the process of cutting the liver parenchyma, the ICG fluorescence real-time navigation is used to continuously correct the cut line to ensure sufficient resection margins. All blood vessels and bile ducts encountered along the way should be disconnected reasonably according to the diameter of the pipe. If intraoperative bleeding cannot be controlled, it should be promptly transferred to open surgery.

In the control group, the first hepatic hilum or the liver parenchyma was dissected and the corresponding liver segment or subsegment Glisson system was selected, then clipped and then cut off; the liver parenchyma was cut off along the ischemic line of the liver surface; and the corresponding liver segment was anatomically resected. The Pringle method was used to block the first hepatic portal during the cutting of the liver parenchyma. If an anatomical hepatectomy is not possible, intraoperative color Doppler ultrasound is used to delineate the resection margin of the liver at a distance of 1–2 cm from the tumor boundary to remove the tumor.

2.1.4 Observation Indicators

The operation time, intraoperative blood loss, the number of new lesions found during the operation, and the postoperative liver function [including alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin (TBIL), and serum albumin (ALB)] and

TABLE 1 | Preoperative clinical features between the ICG-guided and traditional groups.

Characteristics		ICG group	Traditional group	t/χ^2 value	*P*-value
Gender	Male	43	50	0.871	0.351
	Female	5	10		
HBsAg	Positive	40	41	3.200	0.074
	Negative	8	19		
AFP (ng/ml)	≥400	15	23	0.587	0.444
	<400	33	37		
Child–Pugh	A	36	46	0.041	0.840
	B	12	14		
Age (years)		57.3 ± 9.7	56.3 ± 12.1	0.466	0.642

postoperative complications (postoperative bile leakage, bleeding, encapsulated effusion, etc.) were observed and compared.

2.2 Meta-Analysis

2.2.1 Literature Search

Using laparoscopic, hepatectomy, indocyanine green, fluorescence, laparoscopic, hepatectomy, and fluorescence as search terms, the CNKI, Wanfang, Weipu, Cochrane Library, PubMed, Embase, and Web of Science databases were searched, and the search time was limited from the establishment of the databases to October 1, 2021. The references of the included documents were retrospectively searched to obtain and supplement the information not found in the search.

2.2.2 Inclusion and Exclusion Criteria

The inclusion criteria were as follows: 1) The subjects included in the study had liver cancer and underwent laparoscopic hepatectomy; 2) the surgical methods included in the study were both ICG fluorescence navigation laparoscopic hepatectomy and traditional laparoscopic hepatectomy, and a comparison of the curative effects of the two surgical methods was conducted; and 3) the outcome indicators of the study include at least one of the following: R0 resection rate, operation time, intraoperative blood loss, postoperative hospital stay, and postoperative complications.

The exclusion criteria were as follows: 1) studies where the full text cannot be obtained or the specific values of the required indicators cannot be obtained; 2) studies that do not include the comparison results of the two surgical methods; 3) repeated publications; and 4) case reports, conference report, and summary or experimental paper.

2.2.3 Data Extraction and Quality Assessment

Two researchers independently screened and read the literature, extracted the data, and scored the quality. In case of inconsistency, a discussion or negotiation together with a third researcher to assist in the solution was conducted. The data extraction content mainly includes the following: first author, year of publication, journal, country, surgical method, and observation indicators. The observation indicators include R0 resection rate, operation time, intraoperative blood loss, postoperative hospital stay, postoperative complications, etc. The Newcastle-Ottawa Scale (NOS) was used to evaluate the quality of the included literature, and NOS score ≥6 was considered high-quality literature.

2.2.4 Statistical Processing

Clinical data were analyzed by statistical package SPSS 16.0 (SPSS Inc., Chicago, IL, USA). Continuous variables were expressed as the mean ± SE and compared using Student's *t*-test, and non-normally distributed variables were compared using the rank-sum test. Categorical variables were compared using either the χ^2 test or Fisher's exact test, as deemed appropriate. A *P*-value less than 0.05 was considered as statistically significant.

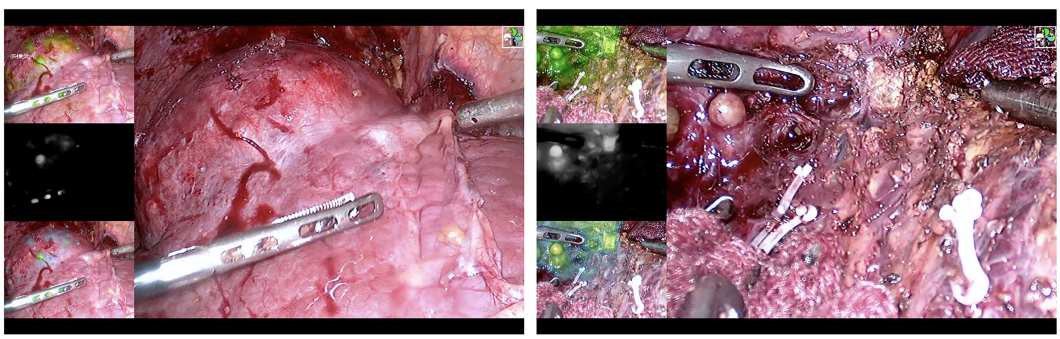

FIGURE 1 | Positive staining. Liver resection under ICG navigation step by step.

Meta-analysis was performed using RevMan 5.3 statistical software. Binary variables were analyzed by odds ratio (OR), whereas continuous variables were analyzed by mean difference (MD). In the statistical analysis, 95% confidence interval (CI) was calculated. The heterogeneity between the included studies was analyzed by χ^2 test and determined by I^2 quantitative analysis: if I^2 <50% and P >0.05, it is considered that the included studies do not have statistical significance; if $P \leq 0.05$ and $I^2 \geq 50\%$, it is judged that the included studies have statistical significance. The publication bias of the included literature studies was analyzed using a funnel chart.

3 RESULTS

3.1 Comparison of Clinicopathological Characteristics Between the Two Groups

There was no significant difference in liver function, platelet, and prothrombin time in the preoperative situation between the two groups (**Table 2**). Considering the general condition and tumor size of the patients, a total of 108 patients were able to tolerate the surgery, and the difference was not statistically significant. The operation time and intraoperative blood loss of the FGLH group were less than those of the LH group (**Table 3**).

The liver function indexes of 48 patients in the FGLH group were significantly better than those of 60 patients in the control group on the first postoperative day, and WBC was lower in the guided group. The incidence of postoperative complications (including postoperative bile leakage, bleeding, and effusion) in the FGLH group was 10/48, compared to 21/60 in the control group. The R0 resection rate in the guided group was 100%, which is higher than the 95.0% (57/60) in the control group, and the postoperative hospital stay was shorter. Fluorescence navigation did not increase the cost, which may be related to the reduction of complications (**Table 4**).

3.2 Meta-Analysis

3.2.1 Included Literature

A total of 873 related studies from the literature were retrieved, and 17 studies were finally included after deletion and selection, all of which were retrospective cohort studies. The flowchart and the results of literature screening are shown in **Figure 2**. The cumulative sample size was 1,441 cases, consisting of 643 cases in the fluorescence laparoscopy group and 798 cases in the traditional laparoscopy group. The basic information of the included literature is shown in **Table 5**.

3.2.2 Meta-Analysis Results

(1) R0 resection: A total of 9 studies compared the R0 resection rate of the fluorescence laparoscopy group and the traditional laparoscopy group, with a total of 707 cases. There was no heterogeneity among the studies (I^2 = 0%, P > 0.05), and the fixed-effects model was used for analysis. The results of the meta-analysis showed that the R0 resection rate between the fluorescence laparoscopy group and the traditional laparoscopy group was significantly different (OR = 3.35, 95% CI: 1.93~5.82, P < 0.0001), as shown in **Figure 3**.

(2) Operation time: A total of 15 studies compared the operation time of the fluorescence laparoscopy group and the traditional laparoscopy group, with a total of 1,208 cases. There was heterogeneity among the studies (I^2 = 80%, P < 0.05), and the random-effects model was used for analysis. The results of the meta-analysis showed that the difference in operation time between the fluorescence laparoscopy group and the traditional laparoscopy group was statistically significant (MD = −15.26, 95% CI: −19.70~−10.82, P < 0.05), as shown in **Figure 4**.

(3) Intraoperative blood loss: A total of 10 studies compared the intraoperative blood loss of the fluorescence laparoscopy group and the traditional laparoscopy group, with a total of 810 cases. The homogeneity of the studies was good (I^2 = 49%, P = 0.04), and the fixed-effects model was used for analysis. The results of the meta-analysis showed that intraoperative blood loss between the fluorescence laparoscopy group and the traditional laparoscopy group was significantly different (MD = −48.65, 95% CI: −53.07~−44.22, P < 0.00001), as shown in **Figure 5**.

(4) Postoperative hospital stay: A total of 10 studies compared the postoperative hospital stay of the fluorescence laparoscopy group and the traditional laparoscopy group, with a total of 769 cases. The homogeneity of the studies was good (I^2 = 18%, P > 0.05), and the fixed-effects model was used for analysis. The results of the meta-analysis showed that the difference in hospital stay between the fluorescence laparoscopy group and the traditional laparoscopy group was statistically significant (MD = −1.15, 95% CI: −1.51~−0.79, P < 0.000001), as shown in **Figure 6**.

(5) Postoperative complications: A total of 14 studies compared the postoperative hospital stays of the fluorescence laparoscopy group and the traditional laparoscopy group, with a total of 1,198 cases. There was no heterogeneity among the studies (I^2 = 0%, P > 0.05), and the fixed-effects model was

TABLE 2 | Preoperative liver function index between the ICG-guided and traditional groups.

Characteristics	ICG group	Traditional group	t/χ^2 value	*P*-value
ALT	35.6 ± 29.8	33.6 ± 20.7	0.404	0.687
AST	34.1 ± 38.7	32.5 ± 13.9	0.295	0.769
TB	15.8 ± 5.7	16.0 ± 7.2	–0.202	0.840
ALB	43.1 ± 3.3	42.6 ± 3.4	0.797	0.427
PLT	155.9 ± 65.4	155.3 ± 63.7	0.052	0.958
PT	11.5 ± 0.8	11.3 ± 1.1	1.259	0.211

TABLE 3 | Intraoperative index between the ICG-guided and traditional groups.

Characteristics	ICG group	Traditional group	t/χ^2 value	*P*-value
Tumor size (cm)	4.6 ± 2.5	5.3 ± 2.7	−1.428	0.156
Blood (ml)	307 ± 214	452 ± 401	−2.259	0.026
Operation time (min)	232 ± 61	279 ± 133	−2.253	0.026

used for analysis. The results of the meta-analysis showed that the postoperative complications of the fluorescence laparoscopy group and the traditional laparoscopy group were statistically significant (OR = 0.63, 95% CI: 0.45~0.88, $P < 0.05$), as shown in **Figure 7**.

3.2.3 Publication Bias Analysis

Publication bias was evaluated by making a funnel chart. The funnel chart results showed that the scattered points were in the funnel chart and were roughly symmetrically distributed, suggesting that the included studies had no obvious publication bias and had little influence on the results of the meta-analysis (**Figure 8**).

4 DISCUSSION

Liver cancer is the sixth most common malignant tumor in the world, and more than half of the newly diagnosed patients are in China (28). Surgical resection is still the best choice for patients with liver cancer (2, 29). No residual tumor (R0) resection of liver tumors is closely related to the prognosis of liver cancer patients. Anatomical hepatectomy is based on the physiological anatomy of the liver and has become the main radical operation for malignant liver tumors, especially tumors in special parts. The core content of the basic concept of precision hepatectomy is to achieve complete eradication of tumors with as little trauma as possible. Therefore, laparoscopic anatomical hepatectomy has become one of the standard treatments for patients with liver cancer.

Laparoscopy technology has the advantages of less trauma and quick recovery (30). At the same time, its unique magnification effect can also assist the surgeon to more clearly identify the tissue structure and various duct systems during the hepatic parenchyma dissection process, improve the efficiency of liver parenchymal dissection, and reduce postoperative complications, thus improving the curative effects and safety of hepatectomy. However, traditional laparoscopic hepatectomy has the following problems: 1) the small tumor cannot be marked in real time during the operation, which makes it difficult to resect multiple small tumors; 2) the resection margin of liver cancer is recommended to be ≥1 cm, and it is difficult to conduct R0 resection by visual observation. 3) During anatomical hepatectomy, it is difficult to adjust the resection plane in real time through the ischemic line, and the liver ducts are not clearly displayed, which may cause complications such as massive bleeding during the operation (31–34). Efficient identification of the boundaries between liver segments is one of the key and difficult points for successful anatomical hepatectomy. At present, the most commonly used method is to ligate the liver pedicle of the target liver segment to form an ischemic line. After the ischemic boundary appears, the liver parenchyma is finely severed. There are obvious limitations in internal discrimination. In addition, the ischemic line is also easily hindered by many factors such as tumor volume, shape, intraoperative bleeding, and tissue scabs, which affect the actual discrimination effect. Therefore, finding a method that can guide and determine the liver segment surface in real time during the entire operation is an important technological breakthrough for laparoscopic anatomical hepatectomy.

ICG is a near-infrared fluorescent dye that can be excited by external light with a wavelength of 750–810 nm, and emits near-infrared light with a wavelength of about 840 nm (35). Because it can be specifically taken up by the liver and excreted through the biliary tract, it does not participate in the characteristics of enterohepatic circulation. Since Ishizawa et al. first reported the application of ICG real-time navigation in laparoscopic hepatectomy in 2009 (36, 37), with the continuous maturity of laparoscopic hepatectomy technology, ICG fluorescence staining technology has gradually been used in laparoscopic anatomical hepatectomy. Injecting ICG to obtain accurate and long-lasting fluorescent staining on the liver surface and parenchyma solves not only the problem of short and easy elution of traditional methylene blue staining but also the problem of the ischemic line caused by adhesions on the liver surface or liver cirrhosis (7, 38, 39).

TABLE 4 | Postoperative index between the ICG-guided and traditional groups.

Characteristics	ICG group	Traditional group	t/χ^2 value	*P*-value
ALT	267.9 ± 135.5	370.7 ± 305.2	−2.338	0.022
AST	260.1 ± 117.1	375.5 ± 317.7	−2.600	0.011
TB	21.4 ± 10.7	22.9 ± 11.5	−0.665	0.508
ALB	36.3 ± 3.4	34.6 ± 3.8	2.357	0.020
WBC	12.4 ± 2.9	13.8 ± 3.3	−2.320	0.022
Hospitalization day	7.6 ± 2.7	8.9 ± 3.5	−2.238	0.027
Cost	40,470 ± 14,790	46,871 ± 51,908	−0.827	0.410

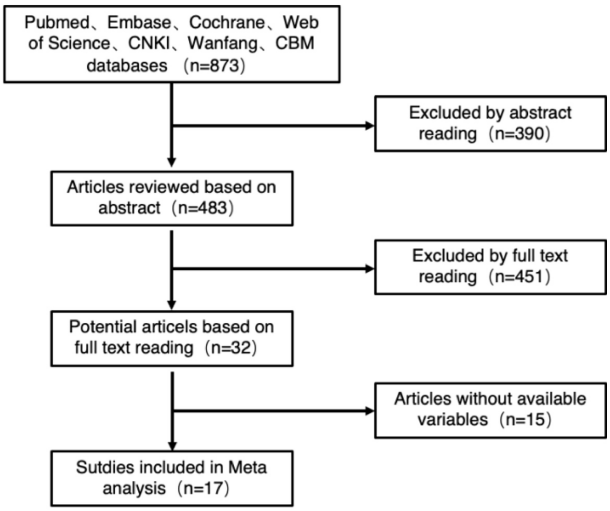

FIGURE 2 | Literature screening flowchart.

This leads to a clear identification and there is no need to block the hepatic artery. Studies have shown that the success rate of ICG fluorescence staining of liver segments can be as high as 95.8%, which is much higher than the traditional ischemic line marking method of 41.7%, and the effect of ICG fluorescence staining is stable and long-lasting (9).

Our study shows that compared with traditional laparoscopic hepatectomy, the application of ICG real-time navigation to laparoscopic hepatectomy can significantly increase the R0 resection rate, shorten the operation time and postoperative hospital stay, and reduce the intraoperative blood loss and postoperative complications. The main reason for the analysis is that ICG fluorescence staining can clarify the three-dimensional boundary of liver cancer and liver anatomical segmentation, guide the segmentation of liver parenchyma throughout the process, increase the possibility of liver cancer R0 resection, and reduce the repeated positioning of intraoperative ultrasound, which greatly shortens the operation time. Since complete standard anatomical hepatectomy was achieved during the operation, unnecessary damage to important blood vessels in the liver and biliary tracts was avoided, the amount of bleeding during the operation was reduced, and the effective liver tissue volume was greatly preserved, so that the remaining liver was replaced to the greatest extent. Consequently, the liver function index recovers quickly after surgery. An increase in white blood cells indicates that the body's homeostasis has been destroyed, and can be used to measure the body's inflammatory response and stress. In this

TABLE 5 | Basic characteristics of the included studies.

Author	Time	Region	ICG group	Traditional group	Characteristics[a]	NOS
Nishino et al. (11)	2017	Japan	23	29	③⑤	8
Aoki et al. (12)	2018	Japan	25	72	②③⑤	8
Chen et al. (13)	2019	China	12	12	①②③④⑤	7
Zhou et al. (14)	2019	China	21	21	①②⑤	8
Xiao et al. (15)	2019	China	67	46	①②⑤	7
Fang et al. (16)	2019	China	23	25	②④	7
Lei et al. (17)	2019	China	36	54	②③⑤	7
Liu et al. (18)	2019	China	24	84	②③④⑤	7
Ma et al. (19)	2019	China	35	40	①②④	7
Lu et al. (20)	2020	China	57	63	①②④	8
Zhang et al. (21)	2020	China	30	34	②③④⑤	8
Pan et al. (22)	2020	China	42	43	①②③④⑤	8
Wang et al. (23)	2020	China	74	74	①⑤	8
Xie and Wu (24)	2020	China	38	65	②③④⑤	7
Zou et al. (25)	2020	China	65	65	②③⑤	7
Wang et al. (26)	2021	China	40	40	①②③④⑤	8
Xin et al. (27)	2021	China	31	31	①②③④⑤	7

[a]*①, R0 resection; ②, operation time; ③, intraoperative blood loss; ④, postoperative hospital stay; and ⑤, postoperative complications.*

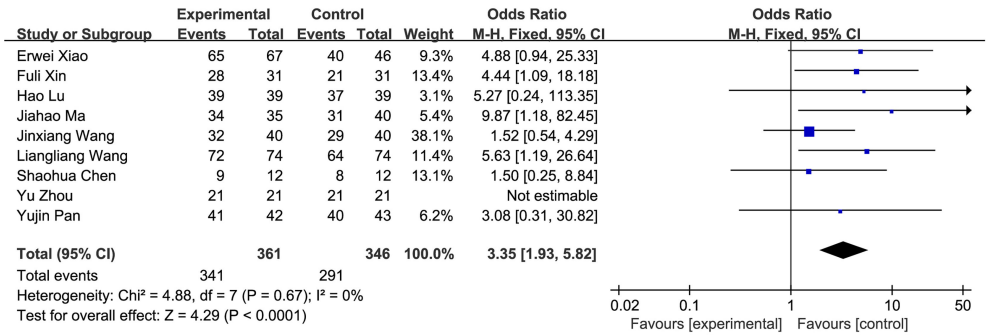

FIGURE 3 | R0 resection between groups with and without ICG fluorescence navigation.

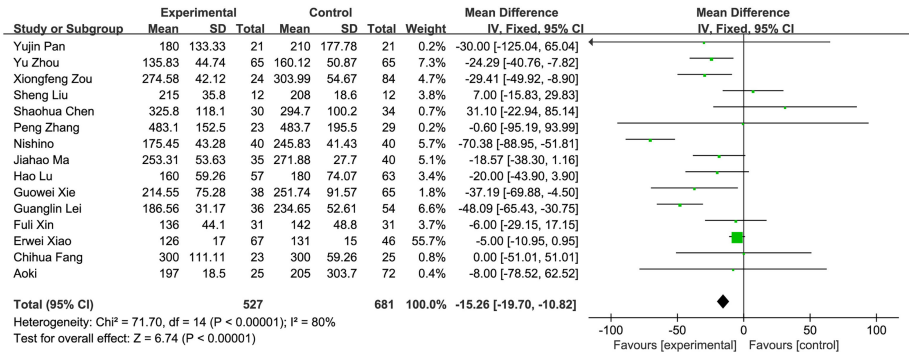

FIGURE 4 | Operation time.

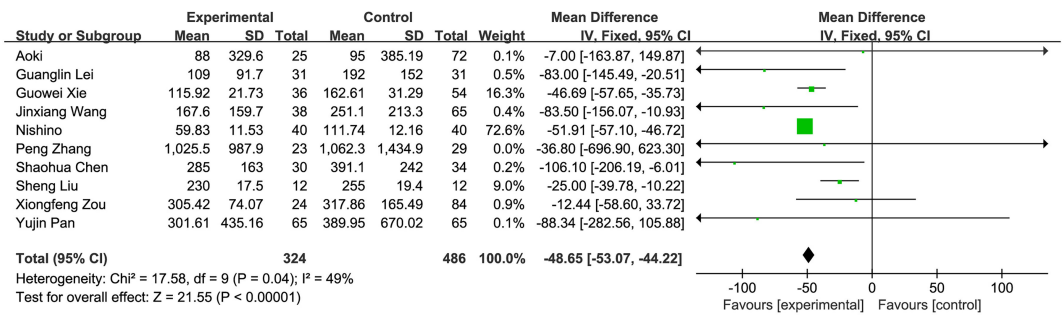

FIGURE 5 | Perioperative bleeding volume.

study, the white blood cells of the observation group decreased compared with those of the control group on the first day after the operation, suggesting that ICG fluorescence imaging has a certain effect in reducing the traumatic shock and postoperative stress response of patients. If there is fluorescent leakage in the bile duct during operation, then timely treatment can greatly reduce the postoperative complications (including postoperative bile leakage, pus, and fever caused by encapsulated effusion), reduce the risk of postoperative liver failure, accelerate the postoperative recovery of the patient, and shorten the postoperative hospital stay (**Figure 9**).

ICG molecular fluorescence imaging technology still has defects in the positioning of deep lesions because the penetration depth of fluorescence is about 5–10 mm (40).

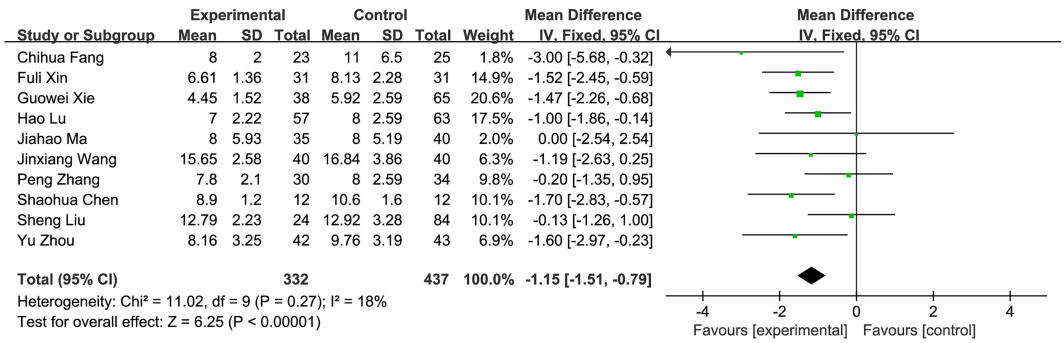

FIGURE 6 | Postoperative hospital stay.

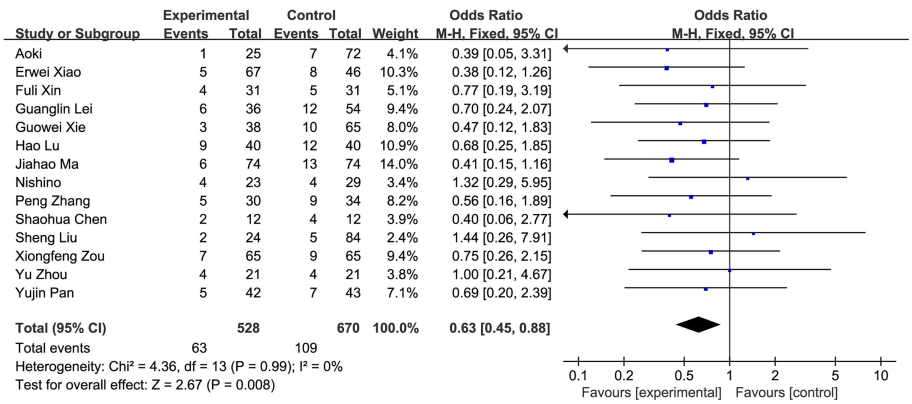

FIGURE 7 | Comparison of postoperative complications.

When ICG fluorescence imaging technology is combined with intraoperative ultrasound, which is difficult to be used for real-time navigation, they can complement each other to achieve a joint effect, and the detection sensitivity can even reach 100%. It has broad application prospects in invasive surgery.

This study also collected relevant literature, systematically evaluated the effectiveness and safety of ICG molecular fluorescence imaging technology in the accurate diagnosis and treatment of liver tumors, and provided reliable evidence-based medical evidence for its widespread clinical promotion. Although this article strictly screened the included literature and formulated the inclusion and exclusion criteria, there were still problems such as the following: 1) the included studies are mostly retrospective cohort studies, and retrospective studies have certain interference factors, which may lead to bias in the research results; 2) different research centers have not performed statistics on the liver segment and pathological stage of the liver cancer patients, so subgroup analysis cannot be performed; 3) most of the literature is for Asian populations and may have an impact on the extrapolation of results; and 4) there is a lack of long-term follow-up data to evaluate long-term efficacy. Therefore, a large sample, multicenter, prospective randomized controlled experiment is still needed to further verify the study.

In the development of ICG imaging-guided techniques for hepatectomy, there is no consensus on the time and dose of preoperative application, and cirrhotic nodules often lead to false positives. The application of ICG dose and timing control requires more clinical data analysis and the development of quantitative indicators to better guide clinical practice. Some scholars believe that intravenous injection of 0.5 mg/kg of ICG within 3 days before surgery can make the lesions show better fluorescence staining during the operation, but some scholars believe that intravenous injection of 7.5 mg of ICG 1 day before surgery also can achieve satisfactory fluorescence display. Our experience is that the preoperative administration time should be adjusted appropriately according to the individual's degree of cirrhosis: for patients without cirrhosis background, ICG is generally administered intravenously at 0.5 mg/kg 2 to 3 days

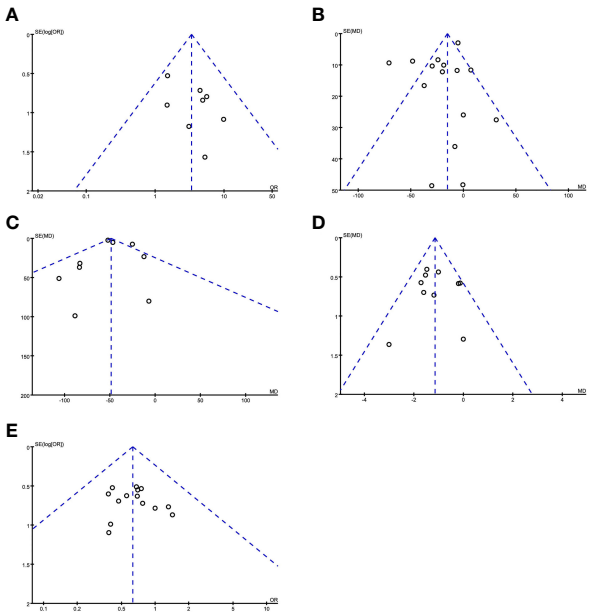

FIGURE 8 | Evaluating publication bias by a funnel chart. **(A)** R0 resection; **(B)** operation time; **(C)** intraoperative blood loss; **(D)** postoperative hospital stay; **(E)** postoperative complications.

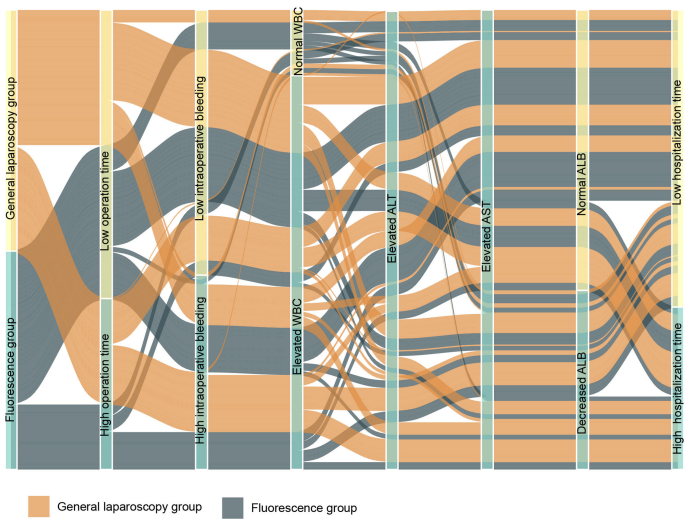

FIGURE 9 | Sankey diagram showing the statistical differences between the two groups.

before surgery; for severe cirrhosis patients, the preoperative injection time can be extended to 7–10 days. Dyeing methods can be divided into positive and negative staining. No matter which staining method is used, the ultimate goal is to distinguish the resected and the retained liver tissue according to the anatomical structure and to guide the operator to better perform anatomical liver resection. Our previous result also suggested that there is no significant difference between positive and reverse stains during liver resection (41).

5 CONCLUSION

ICG molecular fluorescence imaging technology navigation for laparoscopic liver cancer resection is better than conventional

laparoscopic hepatectomy, the operation time is shorter, the intraoperative blood loss is less, the surgical trauma is relatively small, the postoperative hospital stay is shorter, and the perioperative operation is better. The long-term benefits are in line with the concept of accelerated rehabilitation surgery, and its safety and effectiveness are worthy of recognition. Future studies need to further demonstrate the impact of this technology on the long-term survival of patients on the basis of more cases and follow-up time. Although this technology is still in the exploratory stage, it has realized the "radical, anatomical, and functional" resection of liver cancer. This minimally invasive and precise technology has brought the treatment of liver cancer into a new era.

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 study was approved by the Hospital Ethics Committee (Ethics approval no. 2017 Lunxun No. 114). The patients/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

HC, YW, and ZX collected the related data and completed the manuscript and figures. LZ did the statistical analysis. YG, JY, CZ, and WJ did the operations. JM and WL gave constructive guidance and made critical revisions of the manuscript. HC and YW participated in the design of this paper. All authors read and approved the final manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found below:

Supplementary Video 1 | An laparoscopic hepatectomy for treatment of a hepatocellular carcinoma patient using ICG fluorescence positive staining navigation is shown. You can download in this link: https://pan.baidu.com/s/1GS4PLg901G6ahW2Ox-ClqA?pwd=8fa3; Code: 8fa3.

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