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ORIGINAL RESEARCH

Clinical Outcomes of AFP-Negative Patients with Advanced HCC: A Propensity-Matched Analysis from a Retrospective Cohort Study

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Introduction: AFP positivity (≥20 ng/mL) is often used as one of the diagnostic criteria for HCC. The aim of this study is to analyze the prognosis of advanced HCC with negative (<20 ng/mL) AFP at baseline following systemic drug treatment.

Methods: In this study, 91 patients with AFP-negative advanced HCC who received systemic drug treatment in Nanjing Jinling Hospital from February 2011 to September 2023 were collected, and 213 patients with AFP-positive advanced HCC were collected as the control group. A propensity score model was used to adjust for potential confounding variables. Cox regression analysis was used to clarify the differences of prognosis in subgroups for HCC patients.

Results: Following propensity score matching with 1:2 ratio, 90 HCC patients from Group A (AFP-negative) and 180 from Group B (AFP-positive) were chosen to participate in the final analysis set. The OS of AFP-negative HCC patients was extended by 13.5 months compared to AFP-positive HCC patients. Within the AFP-negative HCC group, the top-ranked first-line treatment options were TKIs combo ICIs (mPFS = 9.5m, mOS = 37.1m), chemotherapy combo ICIs (mPFS = 8.1m, mOS = 15.5m), and TKIs (mPFS = 5.6m, mOS = 28.2m). Subgroup analysis indicated that among AFP-negative HCC patients, those without PVTT or with HBV DNA <50lU/mL had longer survival time. For HCC patients who opted for TKIs combo ICIs as their first-line treatment and then switched to TKIs alone for second-line treatment, the mOS and 95% CI were 30.7 (24.8-NA) months.

Conclusion: The survival time of AFP-negative HCC patients was significantly longer than that of AFP positive HCC patients. Patients with no PVTT or HBV DNA <50lU/mL have relatively better efficacy of systemic drug therapy. With the AFP-negative HCC patients, TKIs combo ICIs are preferentially recommended for the first-line therapy, and TKIs are used for the second-line therapy after progression.

Keywords: hepatocellular carcinoma, alpha-fetoprotein, systemic anti-tumor therapy, immune checkpoint inhibitors, multi-line therapy

Introduction

Globally, primary liver cancer (PLC) stands as one of the most prevalent malignant tumors. There were 906,000 new cases of liver cancer globally each year, ranking sixth among all malignant tumors; 830,000 deaths per year, ranking third in 2021.[1,2] Hepatocellular carcinoma (HCC) is the primary pathological type of PLC, accounting for 85% to 90%.[3,4] In China, the primary cause of HCC is chronic infection with the hepatitis B virus (HBV).[5–7] Currently, systemic drug therapy was the primary clinical treatment chosen for advanced HCC, but the prognosis remains unpromising.[8,9] Alpha-fetoprotein (AFP) is a key screening tool for liver cancer recommended by guidelines around the world,[10] and also used for evaluating the efficacy of clinical treatment of HCC.[11,12] Serum AFP ≥20 ng/mL was considered as a reliable indicator for the diagnosis of HCC. However, about 30% of HCC are AFP-negative (<20 ng/mL).[13]

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The mechanism of AFP no-expression is unclear and may be attributed to the silencing of AFP promoter due to mutation or inhibition.[14] The driver mutation gene of AFP-negative liver cancer may be associated with the Wnt/β-catenin pathway or TERT promoter mutations, but the mechanism remains unclear.[15] Abnormal DNA methylation could serve as a significant mechanism underlying AFP negativity.[16] For instance, hypermethylation of tumor suppressor genes or hypomethylation of proto-oncogenes may suppress AFP expression. Additionally, LINC00853 as a lncRNAs, exhibit high expression in AFP-negative liver cancers, potentially inhibiting AFP production by regulating post-transcriptional modifications.[17] AFP-negative HCC may exhibit distinct immune micro-environment characteristics, including variations in PD-L1 expression levels or tumor-infiltrating lymphocytes (TILs).[18] Furthermore, AFP negativity in HBV-related liver cancer might be linked to micro-environment of HBV infection, gene mutations or DNA integration, but the mechanism remains to be further validated.[19]

Currently, none of the existing large-scale Phase III clinical trials of advanced HCC clearly distinguish AFP status, leading to a lack of evidence-based support for the prognosis of AFP-negative patients. For instance, it remains to be verified whether systemic drug treatment, such as tyrosine kinase inhibitors (TKIs), chemotherapy, and TKIs combined with immune checkpoint inhibitors (ICIs), yields similar efficacy in the AFP-negative subgroup as it does in the AFP-positive subgroup.[20,21] At present, some studies have shown that the clinical characteristics of AFP-negative HCC are significantly different from those of AFP-positive patients, such as smaller size, more intact tumor envelope, better pathological stage, and better prognosis.[22,23] In terms of treatment strategies, AFP-negative patients may be more suitable for surgical resection or local treatment because of their relatively indolent tumor biological behavior.[23] However, most of the existing studies focus on AFP-positive patients, and there is still a lack of prospective clinical trials for AFP-negative groups.[12] Therefore, the efficacy of systemic drug therapy for patients with AFP-negative HCC needs to be clarified, and more evidence is urgently needed to optimize treatment decisions and provide data support for the update of liver cancer treatment guidelines.

Materials and Methods

Patients

The study subjects were patients with advanced HCC who underwent systemic drug therapy at the Department of Oncology, Nanjing Jinling Hospital, between February 2011 and September 2023. A cohort study design was employed to recruit AFP-negative (<20 ng/mL) HCC patients as the study group, while the control group included AFP-positive (≥20 ng/mL) HCC patients. Additionally, efforts were made to enhance the collection of patients' subsequent diagnostic and treatment information, as well as to closely monitor their progress. This research protocol adhered to the ethical principles outlined in the 2013 Declaration of Helsinki. The study was approved by the Jinling Hospital Ethics Committee, with the approval number DZQH-KYLL-23-16. Each included subject signed an informed consent before participating in this study.

The sample size estimation for this study was determined based on a cohort study design, focusing on comparing the overall survival (OS) rates between AFP-negative HCC patients (Group A) and AFP-positive HCC patients (Group B). Given the relatively low proportion of AFP-negative HCC, the study aimed to recruit participants at a 1:2 ratio. With estimated hazard ratio (HR) of 0.60 (Group A vs Group B), an 80% endpoint event occurrence rate, $\alpha=0.05$, and $\beta=0.20$, it was estimated that a minimum of 57 subjects in Group A and 114 subjects in Group B would be required. Follow-up continued until 80% of the anticipated death events were observed. Taking into account instances of loss to follow-up and incomplete data collection, the final sample size included 91 subjects in Group A and 213 in Group B.

Inclusion and Exclusion Criteria

The screening of the target population for this study was conducted strictly in accordance with the established inclusion and exclusion criteria outlined below. The inclusion criteria encompassed: a. Pathologically confirmed HCC or clinically diagnosed advanced primary liver cancer; b. Age ≥18 years, inclusive of both males and females; c. No opportunity for radical surgery; d. Participation in at least one systemic drug treatment; e. Expected survival time ≥12 weeks; f. Provision of informed consent for participation in this research project.

Exclusion criteria included: a. Pathologically confirmed other types of tumors, such as intrahepatic cholangiocarcinoma or mixed liver cancer; b. Failure to complete a full cycle of systemic drug treatment; c. Poor overall health status with short expected survival time; d. Patients who refused to participate in this study.

Baseline Demographic and Clinical Information Collection

After inclusion and exclusion, 304 patients finally entered the analysis set (shown in Figure 1). The baseline demographic and clinical information of the enrolled patients were extracted from the medical records of patients in Nanjing Jinling Hospital, including age, gender, medical record number, contact information, home address, and past history of related liver diseases. The baseline indicators of patients undergoing systemic treatment were collected, such as Alanine aminotransferase (ALT), Aspartate transaminase (AST), total bilirubin (TBIL) and AFP. HBV infection related indicators were recorded, including two pairs of hepatitis B (HBsAg, HBsAb, HBeAg, HBeAb, HBcAb) and HBV DNA. Relevant indicators of liver cancer disease were collected: surgical operation, portal vein tumor thrombus (PVTT) and tumor stage.

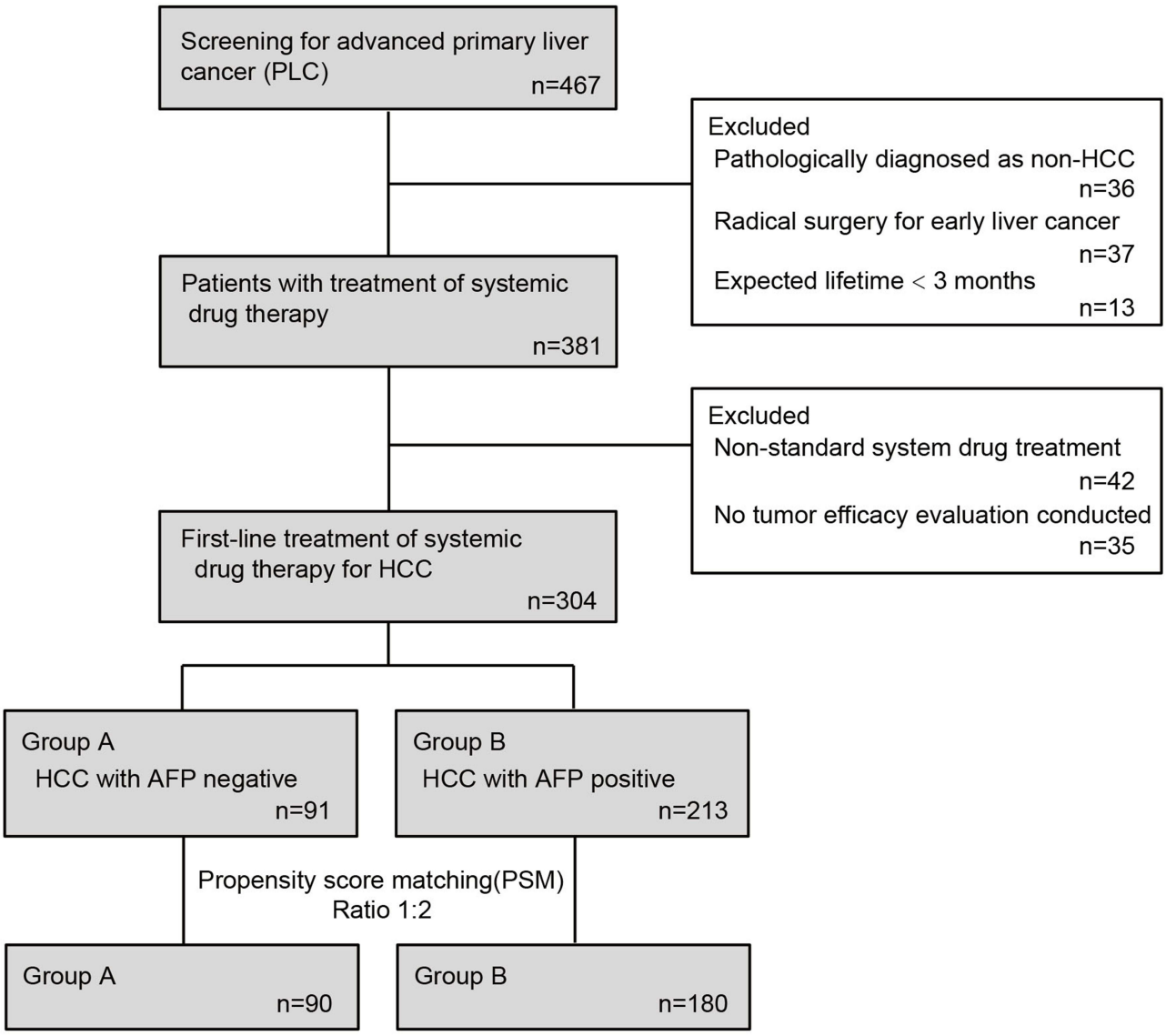

Figure 1 Flow chart of patient selection.

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Follow-Up

In addition to the baseline data of liver cancer patients, the information of systemic drug treatment or local treatment of follow-up patients were also collected. Collect the information of systemic drug treatment, including the treatment plan and drug dose of first-line, second-line and third-line treatment. After the above standardized systemic drug treatment, the tumor of changes for target lesions were evaluated according to RECIST 1.1 criteria. In addition, the duration of systemic drug treatment of patients, including progression-free survival (PFS) and overall survival (OS) were also followed up, which were fully recorded. All the above information should be accurately entered into the computer-related software for subsequent statistical analysis.

Statistical Methods

All collected data were sorted and analyzed by IBM SPSS statistical software (version 25.0 (Armonk, NY, USA; IBM Corporation) and R software (version 4.4.2; R core team). The measurement data are expressed by mean ± standard deviation and analyzed by *t*-test. Count data are expressed as frequency (percentage), and χ^2 test or fisher's exact test was used. The propensity score matching (PSM) method was used to balance the differences between the two groups at baseline, by MathIt package (version 4.7.0). The matching variables included age, gender, ECOG score, Child Pugh score, local treatment, and etiology. All parameters were set as default except "ratio", which was set as 2. Cox regression model was used to analyze the influence of subgroup factors on PFS and OS in HCC patients. Univariate and multivariate analysis were used to determine the independent risk factors affecting the prognosis of the disease. Kaplan–Meier method and hazard ratio (HR) were used to evaluate the influence of risk factors on disease outcome, and Log rank test was used to determine the difference between groups. Land-mark analysis was used to evaluate the effect of treatment intervention at specific time points using jskm R package. Complete case analysis was used to handle missing data. The test cut-off of all statistical analysis in this study was $\alpha = 0.05$.

Results

Basic Information of Included Patients

The baseline characteristics of Group A and Group B were compared before and after PSM to assess potential imbalances. Three hundred and four patients with advanced HCC were finally enrolled in this study, including 91 in group A (AFP-negative) and 213 in group B (AFP-positive). A PSM was used to adjust for potential confounding variables, incorporating some predictors: age, gender, ECOG, Child Pugh, local treatment and etiology (Supplementary Table 1). After PSM, 90 people in group A and 180 people in group B were selected to enter the final analysis set. 77 (85.6%) and 161 participants (89.4%) were male in group A and B, with no statistical difference between the groups ($P > 0.05$).

Before PSM, significant differences were observed in age distribution ($P = 0.004$), tumor stage ($P = 0.003$), presence of PVTT ($P = 0.001$), HBsAg status ($P = 0.024$), and AST positive ($P = 0.007$), indicating notable disparities between the two groups. After PSM, Group A (n = 90) and Group B (n = 180) demonstrated improved balance, though significant differences persisted in age ($P = 0.034$), tumor stage ($P = 0.003$), PVTT ($P = 0.003$), HBsAg status ($P = 0.04$), and AST positive ($P = 0.022$). These factors may be important reasons for AFP negativity. No significant differences were observed in gender, etiology, ECOG performance status, Child-Pugh score, HBV DNA levels, ALT, TBIL, albumin, platelet count, and local treatment before or after PSM ($P > 0.05$). These findings highlight the effectiveness of PSM in reducing baseline imbalances while underscoring the need to account for residual differences in age, tumor stage, PVTT, HBsAg status, and AST levels in subsequent analyses (Table 1). In the post-PSM dataset, only 2 variables, including tumor stage and HBV DNA have missing data. Eleven (4.1%) participants were missing in tumor stage, 18 (6.7%) were missing in HBV DNA.

Effectiveness of HCC Patients with AFP-Negative

By cox regression survival analysis, we compared the differences of PFS and OS between the two groups. The results showed that the median PFS (mPFS) of group A and group B were 6.0 months and 3.9 months, respectively (HR = 0.750, $P = 0.023$) (shown in Figure 2A). The median OS (mOS) of group A was 29.3 months with 95% CI:23.5–37.1 months,

Table 1 Basic Patient Information Before and After PSM

Variables	Before PSM			After PSM		
	Group A (n = 91)	Group B (n = 213)	*P* value	Group A (n = 90)	Group B (n = 180)	*P* value
Gender, n (%)			0.291			0.464
Female	13 (14.3)	20 (9.4)		13 (14.4)	19 (10.6)	
Male	78 (85.7)	193 (90.6)		77 (85.6)	161 (89.4)	
Age, n (%)			0.004			0.034
<50	25 (27.5)	100 (46.9)		25 (27.8)	79 (43.9)	
50~60	36 (39.6)	53 (24.9)		35 (38.9)	51 (28.3)	
≥60	30 (33)	60 (28.2)		30 (33.3)	50 (27.8)	
Etiology, n (%)			0.113			0.163
Alcoholic	7 (7.7)	4 (1.9)		7 (7.8)	3 (1.7)	
HBV	67 (73.6)	157 (73.7)		66 (73.3)	137 (76.1)	
HCV	1 (1.1)	4 (1.9)		1 (1.1)	4 (2.2)	
Unknown	6 (6.6)	12 (5.6)		6 (6.7)	11 (6.1)	
Virus & alcoholic	10 (11)	36 (16.9)		10 (11.1)	25 (13.9)	
Tumor stage, n (%)			0.003			0.003
BCLC A	6 (7.1)	3 (1.4)		6 (7.1)	3 (1.7)	
BCLC B	13 (15.3)	15 (7.2)		13 (15.5)	11 (6.3)	
BCLC C	66 (77.6)	189 (91.3)		65 (77.4)	161 (92)	
PVTT, n (%)			0.001			0.003
No	66 (72.5)	110 (51.6)		65 (72.2)	95 (52.8)	
Yes	25 (27.5)	103 (48.4)		25 (27.8)	85 (47.2)	
ECOG, n (%)			0.698			1
≤1	86 (94.5)	197 (92.5)		85 (94.4)	170 (94.4)	
≥2	5 (5.5)	16 (7.5)		5 (5.6)	10 (5.6)	
Child Pugh, n (%)			0.394			0.746
A	81 (89)	180 (84.5)		80 (88.9)	156 (86.7)	
B	10 (11)	33 (15.5)		10 (11.1)	24 (13.3)	
HBV DNA, n (%)			0.448			0.415
<50IU/mL	52 (62.7)	115 (56.9)		51 (62.2)	95 (55.9)	
≥50IU/mL	31 (37.3)	87 (43.1)		31 (37.8)	75 (44.1)	
HBsAg, n (%)			0.024			0.04
Negative	26 (28.9)	35 (16.7)		26 (28.9)	31 (17.2)	
Positive	64 (71.1)	175 (83.3)		64 (71.1)	149 (82.8)	
HBeAg, n (%)			0.241			0.237
Negative	74 (82.2)	158 (75.2)		74 (82.2)	135 (75)	
Positive	16 (17.8)	52 (24.8)		16 (17.8)	45 (25)	
ALT, n (%)			0.999			0.999
<37U/L	57 (62.6)	134 (62.9)		56 (62.2)	111 (61.7)	
≥37U/L	34 (37.4)	79 (37.1)		34 (37.8)	69 (38.3)	
AST, n (%)			0.007			0.022
<40U/L	57 (62.6)	96 (45.1)		56 (62.2)	84 (46.7)	
≥40U/L	34 (37.4)	117 (54.9)		34 (37.8)	96 (53.3)	
TBIL, n (%)			0.143			0.386
<20.5umml/L	79 (86.8)	168 (78.9)		78 (86.7)	147 (81.7)	
≥20.5umml/L	12 (13.2)	45 (21.1)		12 (13.3)	33 (18.3)	
Albumin, n (%)			0.999			0.399
<35umml/L	14 (15.4)	32 (15)		14 (15.6)	20 (11.1)	
≥35umml/L	77 (84.6)	181 (85)		76 (84.4)	160 (88.9)	
Local treatment, n (%)			0.075			0.481
No	76 (83.5)	156 (73.2)		75 (83.3)	142 (78.9)	
Yes	15 (16.5)	57 (26.8)		15 (16.7)	38 (21.1)	

Notes: PSM factors: age, gender, ECOG, Child Pugh, local treatment, etiology.

Abbreviations: PSM, propensity score matching; HBV, Hepatitis B Virus; HCV, Hepatitis C Virus; BCLC, Barcelona Clinic Liver Cancer; PVTT, portal vein tumor thrombus; ECOG, Eastern Cooperative Oncology Group; HBsAg, Hepatitis B surface Antigen; HBeAg, Hepatitis B e Antigen; ALT, Alanine aminotransferase; AST, Aspartate transaminase; TBIL, total bilirubin.

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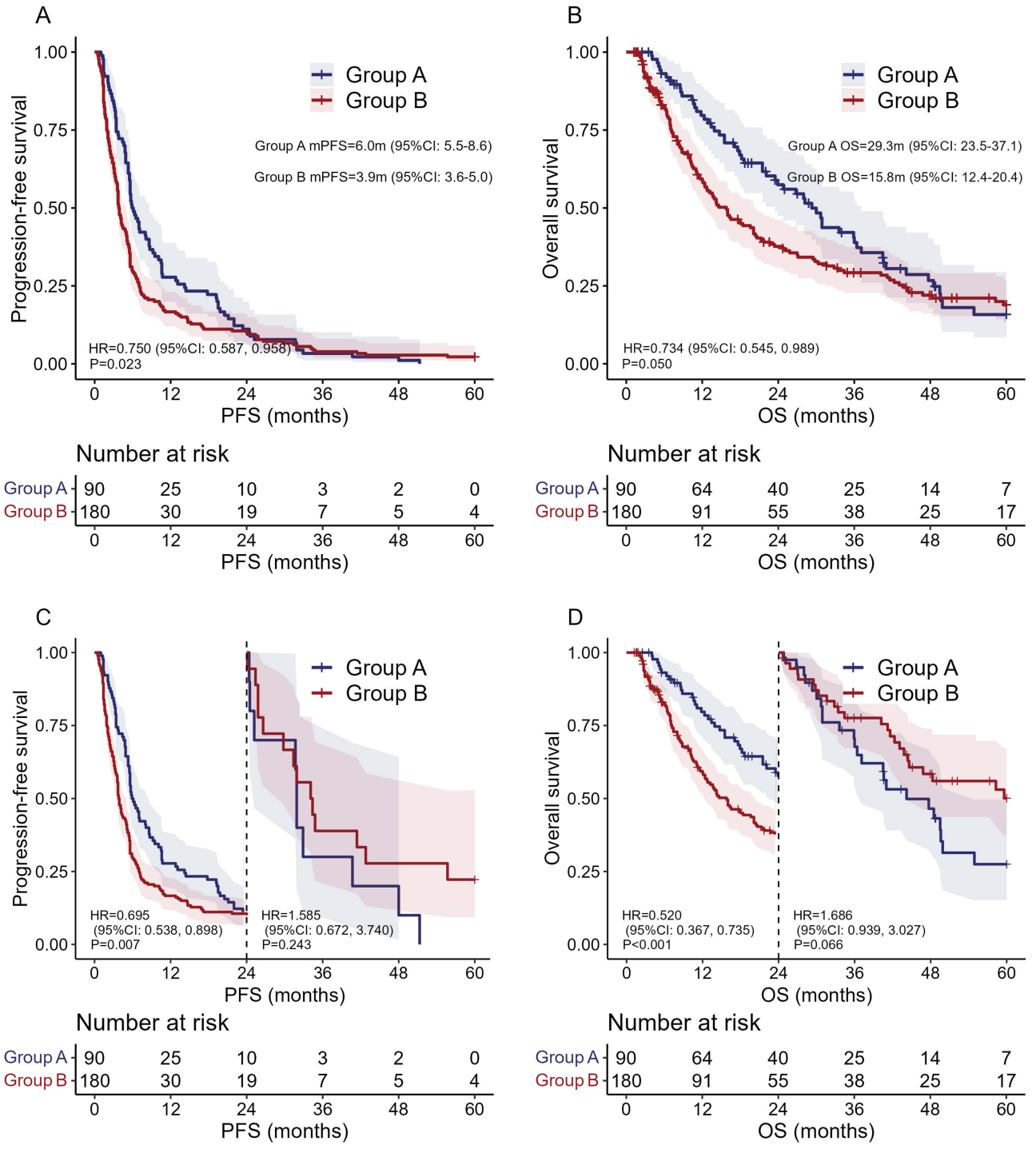

Figure 2 Follow-up information of patients with liver cancer after systemic anti-tumor therapy. (**A**) PFS of HCC patients, (**B**) OS of HCC patients, (**C**) landmark analysis of PFS at 24 months and (**D**) landmark analysis of OS at 24 months. (mPFS, median progression-free survival; mOS, median overall survival; HR, hazard ratio; CI, confidence interval.).

while that of group B was 15.8 months with 95% CI:12.4–20.4 months. The survival time of AFP-negative HCC patients was 13.5 months longer than that of AFP-positive HCC patients (HR = 0.734, P = 0.050)(shown in Figure 2B). Considering the crossover of survival curves, land-mark analysis was used to distinguish the efficacy difference before and after the landmark time of 24 months. In the first 24 months, both the PFS and OS of group A were significantly better than that of group B (HR = 0.695 and 0.469, P = 0.007 and 0.001, respectively) (shown in Figure 2C and D). The

above results show that AFP-negative HCC patients have significant survival advantage compared with AFP-positive HCC patients after systemic drug treatment.

Effect of Different Treatment Strategies on AFP-Negative HCC

The first-line systemic drug treatment options for patients with advanced HCC were as follows: tyrosine kinase inhibitors (TKIs) (37, 41.1% for Group A; 65, 36.1% for Group B), chemotherapy (12, 13.3% for Group A; 41, 22.8% for Group B), immune checkpoint inhibitors (ICIs) (7, 7.8% for Group A; 12, 6.7% for Group B), TKIs combo ICIs (27, 30.0% for Group A; 29, 16.1% for Group B), and chemotherapy combo ICIs (3, 3.3% for Group A; 21, 11.7% for Group B). (Table 2).

Among all the included patients with HCC, the TKIs combo ICIs treatment group had the longest mPFS (7.1 months) and the longest mOS (30.3 months) (shown in Figure 3A and B). In group B, the mPFS and mOS of ICIs alone population were 8.1 months and 43.8 months, respectively (shown in Figure 3C and D). In group A, the order of survival time of each treatment sub-group was as follows: TKIs combo ICIs treatment (mPFS = 9.5m, mOS = 37.1m), Chemotherapy combo ICIS treatment (mPFS = 8.1m, mOS = 15.5m), and TKIs treatment (mPFS = 5.6m, mOS = 28.2m) (shown in Figure 3E and F).

Risk Factors Affecting Prognosis

To analyze the impact of different subgroups on patient survival outcomes, univariate Cox regression analysis was conducted for Group A and B separately. In Group A, PVTT [yes vs no, HR (95% CI):2.09 (1.21, 3.60)] and HBV DNA [>50lU/mL vs <50lU/mL, HR (95% CI): 2.24 (1.29, 3.88)] had significant effect on OS ($p < 0.05$), while Child-Pugh score (B vs A), local treatment (yes vs no), gender (male vs female), age (≥60 vs <50), HBsAg (positive vs negative), ALT (positive vs negative), AST (positive vs negative), TBIL (positive vs negative) had no statistical effect on OS ($P > 0.05$). (Figure 4). In Group B, however, the Child-Pugh score [B vs A, HR (95% CI):1.75 (1.07,2.88)], local treatment [yes vs no, HR (95% CI):0.43 (0.27, 0.70)] and first-line treatment strategy [T2 vs T1, HR (95% CI):2.28 (1.43, 3.64)] had significant effect on OS ($p < 0.05$), while gender (male vs female), age (≥60 vs <50), PVTT (yes vs no), HBV DNA (positive vs negative), HBsAg (positive vs negative), ALT (positive vs negative), AST (positive vs negative), TBIL (positive vs negative) and other factors had no statistical effect on OS ($P > 0.05$) (shown in Supplemental Figure 1).

Sensitivity Analysis: Multi-Line Therapies

Factors influencing the OS of systemic drug therapy in Group A include not only subgroup differences but also variations in treatment strategies. Beyond first-line systemic drug therapy, subsequent multi-line treatments also indirectly impact the OS of HCC patients. After initially opting for TKIs alone to combat tumor progression, 13.0% of Group A patients

Table 2 The Systemic Drug Therapies Between the Two Groups

	Before PSM			After PSM		
	Group A (n = 91)	**Group B (n = 213)**	**P value**	**Group A (n = 90)**	**Group B (n = 180)**	**P value**
First line treatment, n (%)			0.015			0.016
T1	37 (40.7)	77 (36.2)		37 (41.1)	65 (36.1)	
T2	13 (14.3)	48 (22.5)		12 (13.3)	41 (22.8)	
T3	7 (7.7)	14 (6.6)		7 (7.8)	12 (6.7)	
T4	27 (29.7)	34 (16.0)		27 (30.0)	29 (16.1)	
T5	3 (3.3)	24 (11.3)		3 (3.3)	21 (11.7)	
T6	4 (4.4)	16 (7.5)		4 (4.4)	12 (6.7)	

Abbreviations: T1, tyrosine kinase inhibitors (TKIs); T2, Chemotherapy; T3, immune checkpoint inhibitors (ICIs); T4, TKIs combo ICIs; T5, Chemotherapy combo ICIs; T6, Other therapies.

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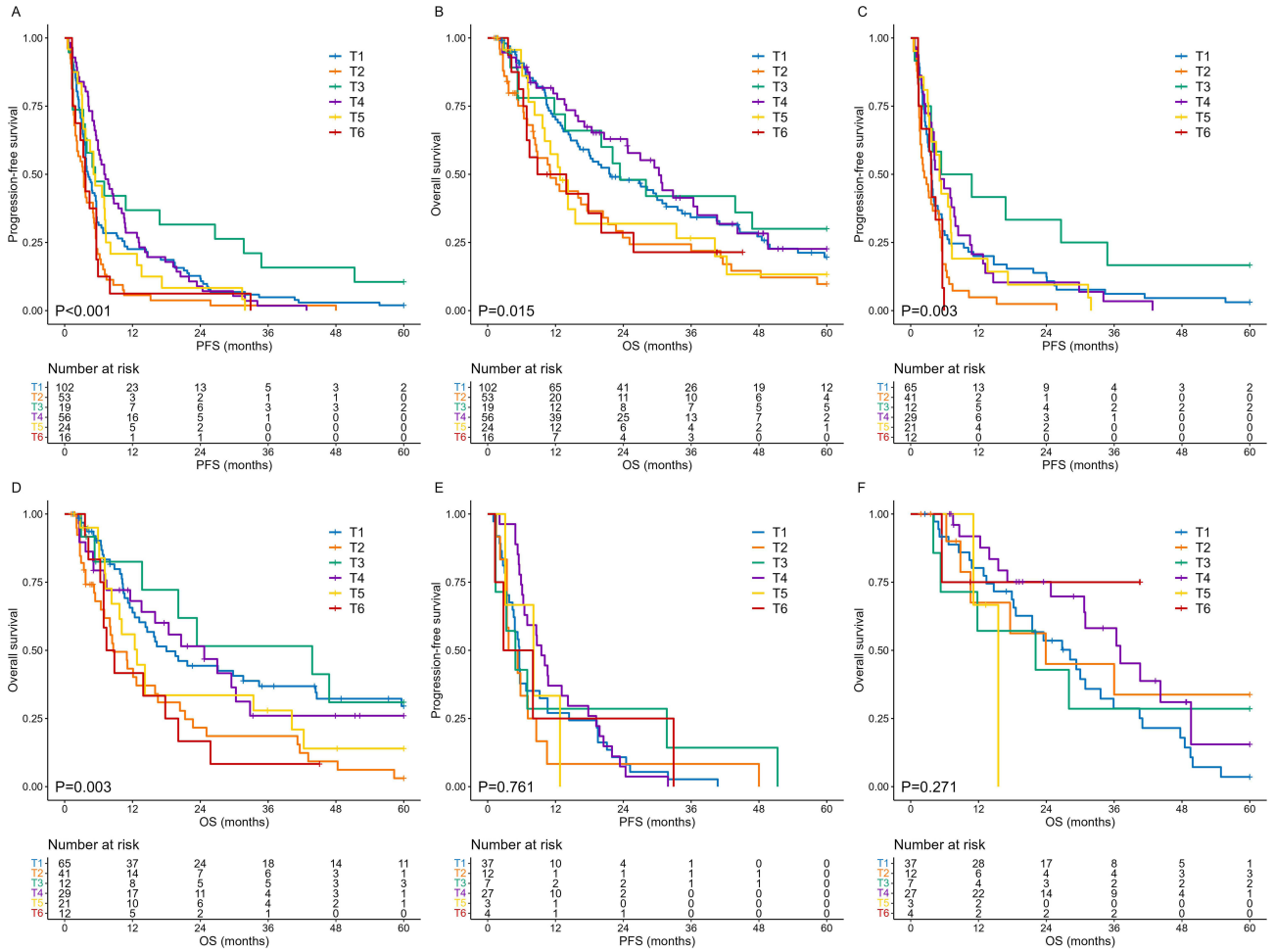

Figure 3 The effectiveness of first-line systemic treatment on patient prognosis. (**A**) PFS of HCC patients; (**B**) OS of HCC patients; (**C**) PFS of HCC patients with AFP positive; (**D**) OS of HCC patients with AFP positive; (**E**) PFS of HCC patients with AFP negative; (**F**) OS of HCC patients with AFP negative.

chose TKIs combo ICIs treatment as the second-line therapy. The mOS (95% CI) was 18.2 (17.9-NA) months. Conversely, for those initially treated with TKIs combo ICIs, 42.9% patients chose TKIs-alone as the second-line therapy. This sequential therapy strategy for HCC patients of the mOS (95% CI) was 30.7(24.8-NA) months in Group A (Figure 5A), and 16.0(2.6-NA) months in Group B (Figure 5B). This clearly demonstrated that AFP-negative HCC patients who receive ICIs earlier tend to have improved OS.

Discussion

HCC is a malignant tumor with high incidence worldwide, and early diagnosis and effective treatment are essential to improve the prognosis of patients. As a classic biomarker of HCC, AFP has long been used in the screening, diagnosis and efficacy monitoring of HCC.[24] However, about 30–40% of HCC patients are negative for serum AFP. Such patients often face the challenges of diagnosis delay and treatment options due to the lack of specific biomarkers.[25,26] The clinical characteristics of AFP-negative HCC are significantly different from those of AFP-positive patients.[27,28] However, most of the existing studies focus on AFP-positive HCC patients. Prospective clinical trials for AFP-negative HCC patients are still lacking, and more evidence-based medical evidence is urgently needed.

This research team had published an article about the prognostic efficacy of young liver cancer patients (<35 years old).[29] The efficacy of systemic anti-tumor therapy in young patients was poorer compared with that in elderly patients. Young patients with HCC had a high HBV infection rate and were prone to hyperprogressive disease (HPD).[29] Whereas, this study prioritizes elucidating the effects of multi-line therapies on overall survival (OS). Based on this study, we significantly increased the sample

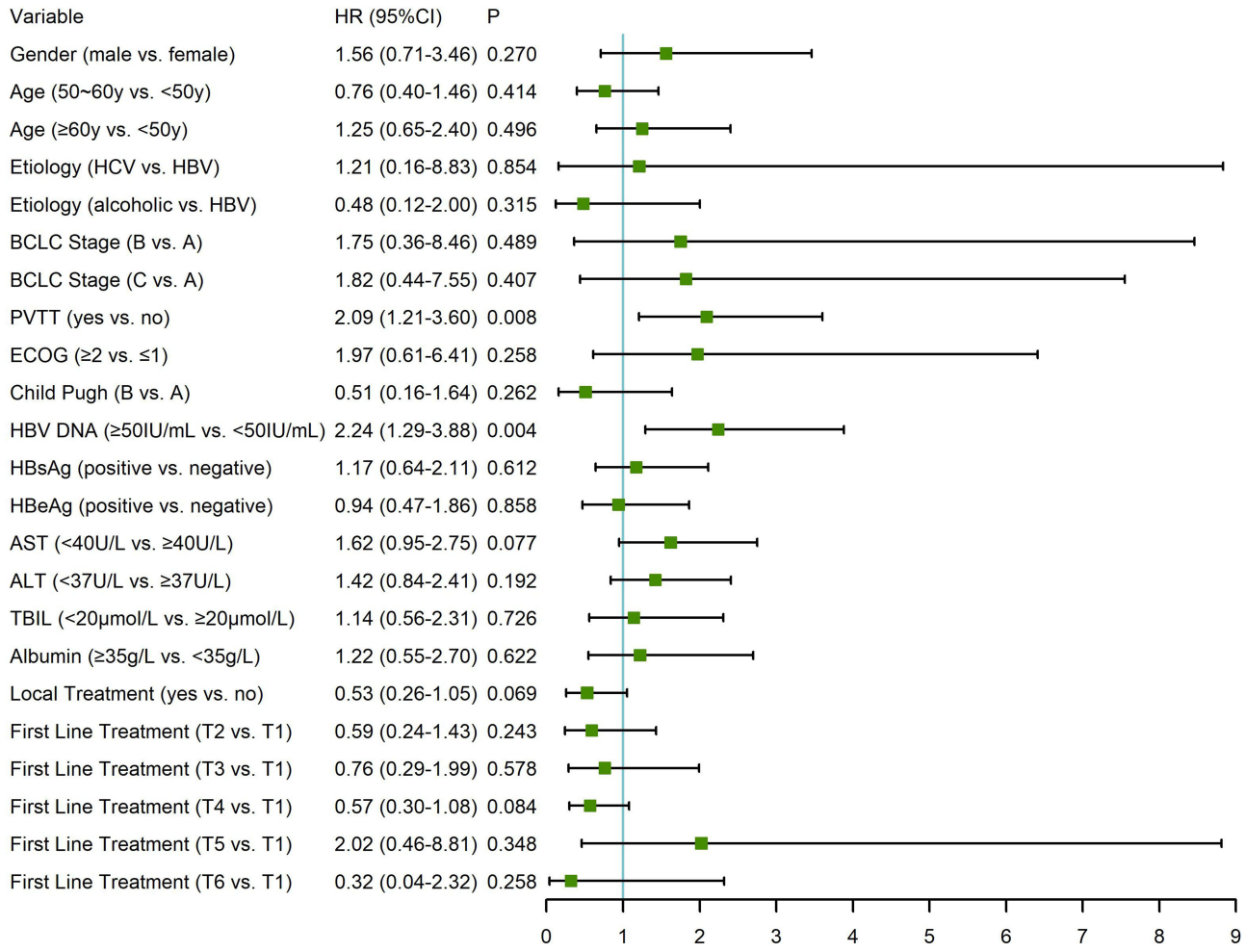

Figure 4 Forest plot of OS for HCC patients with AFP negative.

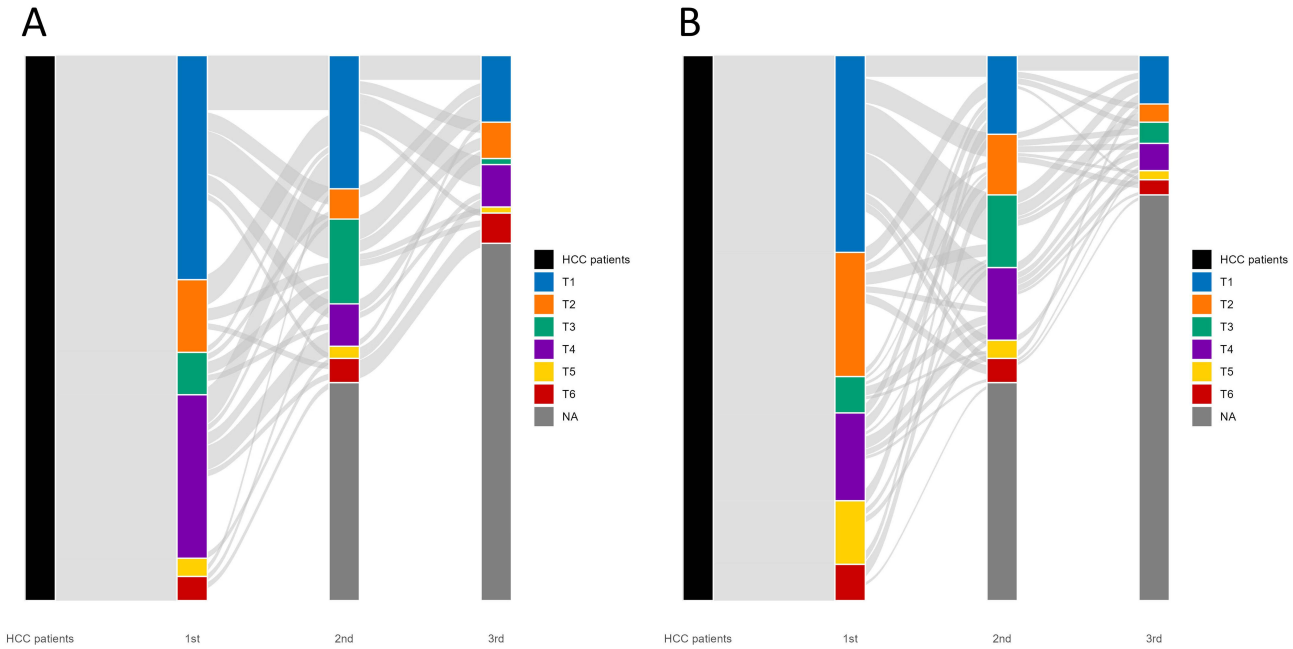

Figure 5 Multi-line systemic therapies on HCC patients. (**A**) multi-line systemic therapies on HCC patients with AFP-negative. (**B**) multi-line systemic therapies on HCC patients with AFP-positive.

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size and conducted exploratory analyses for AFP-negative liver cancer, a characteristic group. AFP-negative HCC may have different therapeutic effects due to variations in tumor angiogenesis mechanisms.[30] AFP-negative HCC may exhibit different drug sensitivities due to tumor heterogeneity. For example, the atezolizumab and bevacizumab combination regimen in the IMbrave150 trial significantly extended survival but the efficacy in the AFP-negative subgroup were not clarified.[21] Therefore, this innovative study focused on the clinical characteristics and prognosis of AFP negative HCC patients.

This study found that the mOS of AFP-negative HCC patients was 29.3 months, 95% CI:23.5–37.1 months, which superior to HCC patients of most clinical research trials. Factors affecting the efficacy of systemic drug therapy in patients with AFP-negative HCC, in addition to subgroups, there are also differences in the efficacy of various treatment strategies.[23] In addition to first-line systemic drug therapy, subsequent multi-line therapy also indirectly affected the OS of patients. This study found that in patients with AFP-negative HCC, after the first-line choice of using TKIs alone for anti-tumor progression, the proportion of patients using TKIs combo ICIs for second-line therapy was 13.0%, and the mOS (95% CI) were 18.2 (17.9-NA) months. However, after the first-line treatment of TKIs combo ICIs for anti-tumor progression, 42.9% of the second-line use of TKIs alone treatment, with a mOS (95% CI) of 30.7 (24.8-NA) months. It fully reflects that the earlier the use of ICIs in patients with AFP-negative liver cancer, the longer the survival time.

This study observed and analyzed the efficacy of systemic treatment in patients with advanced liver cancer, but there are still many deficiencies. This is a single center cohort study, and it is impossible to achieve randomized controlled grouping. The sample size of this study is relatively small, especially for the data of multi-line treatment. The inclusion and exclusion criteria of this study, as well as the follow-up treatment, are all based on real-world research designs, which cannot limit the diagnosis and treatment behavior of patients like clinical drug registration trials. Despite PSM, significant differences remained between groups in key variables including age, tumor stage, PVTT, HBsAg status, and AST levels. These persistent imbalances could confound the analysis of survival outcomes, potentially overestimating the impact of AFP status on prognosis. This study lacks evidence of mechanism studies, and subsequent studies on liver tumor organoid models will help deepen our understanding of the clinical treatment of AFP-negative HCC.[31] It is hoped that the follow-up research can overcome the above shortcomings and finally get a more comprehensive research conclusion.

Conclusion

The survival time of AFP-negative HCC patients is significantly longer than that of AFP-positive HCC patients. Patients with no PVTT or HBV DNA <50lU/mL have longer OS than the control group. Among AFP-negative HCC patients, TKIs combo ICIs are preferentially recommended for the first-line therapy, and TKIs are recommended for the second-line therapy after progression, which has significantly benefit for survival.

Statement of Ethics

The studies involving human participants were reviewed and approved by the Ethics Committee of Nanjing Jinling Hospital (approval no. DZQH-KYLL-23-16).

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

All authors have no conflicts of interest to declare for this work.

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