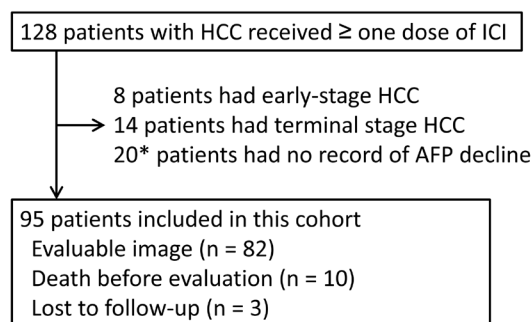


AFP response predicts treatment outcomes of ICI combination therapies



Supplementary Figure 1. Flowchart of patient recruitment in this study. *Nine patients with BCLC stage A or D. AFP, α -fetoprotein; HCC, hepatocellular carcinoma; ICI, immune checkpoint inhibitor.

Supplementary Table 1. TRAEs in 95 patients with hepatocellular carcinoma

Type of TRAE (n=66)	TRAE, n (%)	
	Any grade	Grade ≥ 3
Hepatitis*	25 (26.3)	7 (7.4)
Fatigue	17 (17.9)	1 (1.1)
Dermatitis	14 (14.7)	4 (4.2)
Colitis	11 (11.6)	2 (2.1)
Hand foot syndrome	9 (9.5)	2 (2.1)
Fever	9 (9.5)	1 (1.1)
Pneumonitis*	4 (4.2)	4 (4.2)
Gastric necrosis	1 (1.1)	1 (1.1)
Proteinuria	3 (3.2)	0 (0)
Myalgia	1 (1.1)	0 (0)
Dizziness	1 (1.1)	0 (0)
Edema	1 (1.1)	0 (0)

*Among five patients who died from TRAEs, three and two died from severe hepatitis and pneumonitis, respectively. TRAEs, treatment-related adverse events.

Supplementary Table 2. Sensitivity, specificity, and Youden's index of different declines in the serum AFP level within the initial 3 months of immune checkpoint inhibitor therapy

Cut-off for AFP decline	Sensitivity	Specificity	Youden's index
>10%	0.7255	0.7273	0.4528
>15%	0.7255	0.7955	0.5209
>20%	0.6275	0.8182	0.4456

AFP, α -fetoprotein.

Supplementary Table 3. Factors associated with disease control in 82 patients with HCC who underwent radiological imaging (a decline of >10% in the AFP level)

Character		Univariate analysis		Multivariate analysis	
		OR (95% CI)	P value	OR (95% CI)	P value
Age (year)		1.064 (1.016-1.113)	0.008		
Sex	Male vs. female	0.371 (0.073-1.872)	0.230		
Alcohol	Yes vs. no	0.752 (0.274-2.065)	0.580		
HBV	Yes vs. no	0.452 (0.180-1.133)	0.090		
HCV	Yes vs. no	2.600 (0.848-7.971)	0.095		

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DM	Yes vs. no	1.247 (0.486-3.202)	0.647		
Grade 1-2 TRAEs	Yes vs. no	2.769 (1.103-6.954)	0.030		
Grade \geq 3 TRAEs	Yes vs. no	1.256 (0.345-4.576)	0.730		
TTV (cm ³)	>1000 vs. \leq 1000	0.633 (0.256-1.565)	0.322		
MVI	Yes vs. no	0.336 (0.130-0.870)	0.025		
EHM	Yes vs. no	0.498 (0.192-1.290)	0.151		
AFP (ng/mL)	\geq 400 vs. <400	0.273 (0.107-0.701)	0.007		
AST (U/L)	>40 vs. \leq 40	0.412 (0.142-1.197)	0.103		
ALT (U/L)	>40 vs. \leq 40	0.452 (0.180-1.133)	0.090		
NLR	>3.0 vs. \leq 3.0	0.205 (0.062-0.673)	0.009		
Child-Pugh class	B vs. A	0.757 (0.285-2.010)	0.576		
ALBI grade	2/3 vs. 1	0.571 (0.195-1.673)	0.307		
AFP decline >10%	Yes vs. no	7.145 (2.631-19.404)	0.001	6.163 (1.755-21.640)	0.005
Combination therapy*	Yes vs. no	2.683 (0.769-9.359)	0.121		

*Combination therapy includes tyrosine kinase inhibitors, radiofrequency ablation, transarterial chemoembolization, and stereotactic body radiotherapy for hepatocellular carcinoma. AFP, α -fetoprotein; ALBI, albumin-bilirubin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; DM, diabetes mellitus; EHM, extrahepatic metastasis; HBV, hepatitis B virus; HCV, hepatitis C virus; TRAEs, treatment-related adverse events; MVI, macroscopic vascular invasion; NLR, neutrophil-to-lymphocyte ratio; TTV, total tumor volume.

Supplementary Table 4. Factors associated with disease control in 82 patients with HCC who underwent radiological imaging (a decline of >20% in the AFP level)

Character		Univariate analysis		Multivariate analysis	
		OR (95% CI)	P value	OR (95% CI)	P value
Age (year)		1.064 (1.016-1.113)	0.008		
Sex	Male vs. female	0.371 (0.073-1.872)	0.230		
Alcohol	Yes vs. no	0.752 (0.274-2.065)	0.580		
HBV	Yes vs. no	0.452 (0.180-1.133)	0.090		
HCV	Yes vs. no	2.600 (0.848-7.971)	0.095		
DM	Yes vs. no	1.247 (0.486-3.202)	0.647		
Grade 1-2 irAEs	Yes vs. no	2.769 (1.103-6.954)	0.030		
Grade \geq 3 irAEs	Yes vs. no	1.256 (0.345-4.576)	0.730		
TTV (cm ³)	>1000 vs. \leq 1000	0.633 (0.256-1.565)	0.322		
MVI	Yes vs. no	0.336 (0.130-0.870)	0.025		
EHM	Yes vs. no	0.498 (0.192-1.290)	0.151		
AFP (ng/mL)	\geq 400 vs. <400	0.273 (0.107-0.701)	0.007		
AST (U/L)	>40 vs. \leq 40	0.412 (0.142-1.197)	0.103		
ALT (U/L)	>40 vs. \leq 40	0.452 (0.180-1.133)	0.090		
NLR	>3.0 vs. \leq 3.0	0.205 (0.062-0.673)	0.009		
Child-Pugh class	B vs. A	0.757 (0.285-2.010)	0.576		
ALBI grade	2/3 vs. 1	0.571 (0.195-1.673)	0.307		
AFP decline >20%	Yes vs. no	7.639 (2.646-22.050)	<0.001	7.802 (2.015-30.207)	0.003
Combination therapy*	Yes vs. no	2.683 (0.769-9.359)	0.121		

*Combination therapy includes tyrosine kinase inhibitors, radiofrequency ablation, transarterial chemoembolization, and stereotactic body radiotherapy for hepatocellular carcinoma. AFP, α -fetoprotein; ALBI, albumin-bilirubin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; DM, diabetes mellitus; EHM, extrahepatic metastasis; HBV, hepatitis B virus; HCV, hepatitis C virus; TRAEs, treatment-related adverse events; MVI, macroscopic vascular invasion; NLR, neutrophil-to-lymphocyte ratio; TTV, total tumor volume.

AFP response predicts treatment outcomes of ICI combination therapies

Supplementary Table 5. Factors associated with progression-free survival in 95 patients with unresectable hepatocellular carcinoma (a decline of >10% in the AFP level)

Character	Univariate analysis		Multivariate analysis		
		HR (95% CI)	P value	HR (95% CI)	P value
Age (year)		0.976 (0.955-0.998)	0.034		
Sex	Male vs. female	1.270 (0.607-2.659)	0.526		
Alcohol	Yes vs. no	2.004 (1.211-3.316)	0.007		
HBV	Yes vs. no	1.107 (0.690-1.777)	0.673		
HCV	Yes vs. no	0.641 (0.365-1.124)	0.120		
DM	Yes vs. no	0.814 (0.495-1.340)	0.419		
Grade 1-2 TRAEs	Yes vs. no	0.572 (0.356-0.919)	0.021		
Grade ≥3 TRAEs	Yes vs. no	1.455 (0.799-2.647)	0.220		
TTV (cm ³)	>1000 vs. ≤1000	1.641 (1.021-2.635)	0.041		
MVI	Yes vs. no	2.193 (1.323-3.635)	0.002	3.355 (1.659-6.788)	0.001
EHM	Yes vs. no	1.375 (0.837-2.260)	0.209		
AFP (ng/mL)	<400 vs. ≥400	2.021 (1.246-3.278)	0.004		
AST (U/L)	>40 vs. ≤40	1.939 (1.094-3.436)	0.023		
ALT (U/L)	>40 vs. ≤40	1.644 (1.011-2.672)	0.045	2.576 (1.148-5.780)	0.022
NLR	>3.0 vs. ≤3.0	2.181 (1.221-3.895)	0.008		
Child-Pugh class	B vs. A	1.659 (1.018-2.704)	0.042		
ALBI grade	2/3 vs. 1	1.649 (0.936-2.905)	0.084		
AFP decline >10%	Yes vs. no	0.314 (0.192-0.514)	<0.001	0.378 (0.200-0.716)	0.003
Combination therapy*	Yes vs. no	0.363 (0.206-0.641)	<0.001	0.249 (0.114-0.543)	<0.001
Best response	CR+PR+SD vs. none	0.112 (0.064-0.198)	<0.001	0.119 (0.052-0.272)	<0.001

*Combination therapy includes tyrosine kinase inhibitors, radiofrequency ablation, transarterial chemoembolization, and stereotactic body radiotherapy for hepatocellular carcinoma. AFP, α-fetoprotein; ALBI, albumin-bilirubin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CR+PR+SD, complete response plus partial response plus stable disease; DM, diabetes mellitus; EHM, extrahepatic metastasis; HBV, hepatitis B virus; HCV, hepatitis C virus; TRAEs, treatment-related adverse events; MVI, macroscopic vascular invasion; NLR, neutrophil-to-lymphocyte ratio; TTV, total tumor volume.

Supplementary Table 6. Factors associated with progression-free survival in 95 patients with unresectable hepatocellular carcinoma (a decline of >20% in the AFP level)

Character	Univariate analysis		Multivariate analysis		
		HR (95% CI)	P value	HR (95% CI)	P value
Age (year)		0.976 (0.955-0.998)	0.034		
Sex	Male vs. female	1.270 (0.607-2.659)	0.526		
Alcohol	Yes vs. no	2.004 (1.211-3.316)	0.007		
HBV	Yes vs. no	1.107 (0.690-1.777)	0.673		
HCV	Yes vs. no	0.641 (0.365-1.124)	0.120		
DM	Yes vs. no	0.814 (0.495-1.340)	0.419		
Grade 1-2 TRAEs	Yes vs. no	0.572 (0.356-0.919)	0.021		
Grade ≥3 TRAEs	Yes vs. no	1.455 (0.799-2.647)	0.220		
TTV (cm ³)	>1000 vs. ≤1000	1.641 (1.021-2.635)	0.041		
MVI	Yes vs. no	2.193 (1.323-3.635)	0.002	3.360 (1.659-6.805)	0.001
EHM	Yes vs. no	1.375 (0.837-2.260)	0.209		
AFP (ng/mL)	<400 vs. ≥400	2.021 (1.246-3.278)	0.004		
AST (U/L)	>40 vs. ≤40	1.939 (1.094-3.436)	0.023		
ALT (U/L)	>40 vs. ≤40	1.644 (1.011-2.672)	0.045	2.576 (1.148-5.780)	0.022
NLR	>3.0 vs. ≤3.0	2.181 (1.221-3.895)	0.008		
Child-Pugh class	B vs. A	1.659 (1.018-2.704)	0.042		

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ALBI grade	2/3 vs. 1	1.649 (0.936-2.905)	0.084		
AFP decline >20%	Yes vs. no	0.417 (0.254-0.682)	0.001	0.430 (0.223-0.829)	0.012
Combination therapy*	Yes vs. no	0.363 (0.206-0.641)	<0.001	0.230 (0.103-0.511)	<0.001
Best response	CR+PR+SD vs. none	0.112 (0.064-0.198)	<0.001	0.126 (0.056-0.283)	<0.001

*Combination therapy includes tyrosine kinase inhibitors, radiofrequency ablation, transarterial chemoembolization, and stereotactic body radiotherapy for hepatocellular carcinoma. AFP, α -fetoprotein; ALBI, albumin-bilirubin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CR+PR+SD, complete response plus partial response plus stable disease; DM, diabetes mellitus; EHM, extrahepatic metastasis; HBV, hepatitis B virus; HCV, hepatitis C virus; TRAEs, treatment-related adverse events; MVI, macroscopic vascular invasion; NLR, neutrophil-to-lymphocyte ratio; TTV, total tumor volume.

Supplementary Table 7. Factors associated with overall survival in 95 patients with unresectable hepatocellular carcinoma (a decline of >10% in the AFP level)

Character		Univariate analysis		Multivariate analysis	
		HR (95% CI)	P value	HR (95% CI)	P value
Age (year)		0.985 (0.959-1.012)	0.266		
Sex	Male vs. female	2.416 (0.751-7.771)	0.139		
Alcohol	Yes vs. no	1.447 (0.777-2.695)	0.244		
HBV	Yes vs. no	1.134 (0.645-1.994)	0.663		
HCV	Yes vs. no	0.727 (0.363-1.455)	0.368		
DM	Yes vs. no	0.646 (0.348-1.199)	0.166		
Grade 1-2 TRAEs	Yes vs. no	0.431 (0.243-0.763)	0.004		
Grade \geq 3 TRAEs	Yes vs. no	1.755 (0.894-3.446)	0.102		
TTV (cm ³)	>1000 vs. \leq 1000	2.247 (1.278-3.950)	0.005		
MVI	Yes vs. no	3.803 (1.961-7.375)	<0.001	4.008 (1.637-9.810)	0.002
EHM	Yes vs. no	1.310 (0.733-2.339)	0.362		
AFP (ng/mL)	<400 vs. \geq 400	3.113 (1.744-5.557)	<0.001		
AST (U/L)	>40 vs. \leq 40	3.510 (1.564-7.876)	0.002		
ALT (U/L)	>40 vs. \leq 40	2.163 (1.208-3.872)	0.009		
NLR	>3.0 vs. \leq 3.0	3.704 (1.657-8.280)	0.001		
Child-Pugh class	B vs. A	2.492 (1.417-4.381)	0.002		
ALBI grade	2/3 vs. 1	3.499 (1.553-7.883)	0.003		
AFP decline >10%	Yes vs. no	0.383 (0.217-0.676)	0.001	0.395 (0.190-0.821)	0.013
Combination therapy*	Yes vs. no	0.441 (0.218-0.892)	0.023		
Best response	CR+PR+SD vs. none	0.165 (0.088-0.308)	<0.001	0.429 (0.203-0.908)	0.027

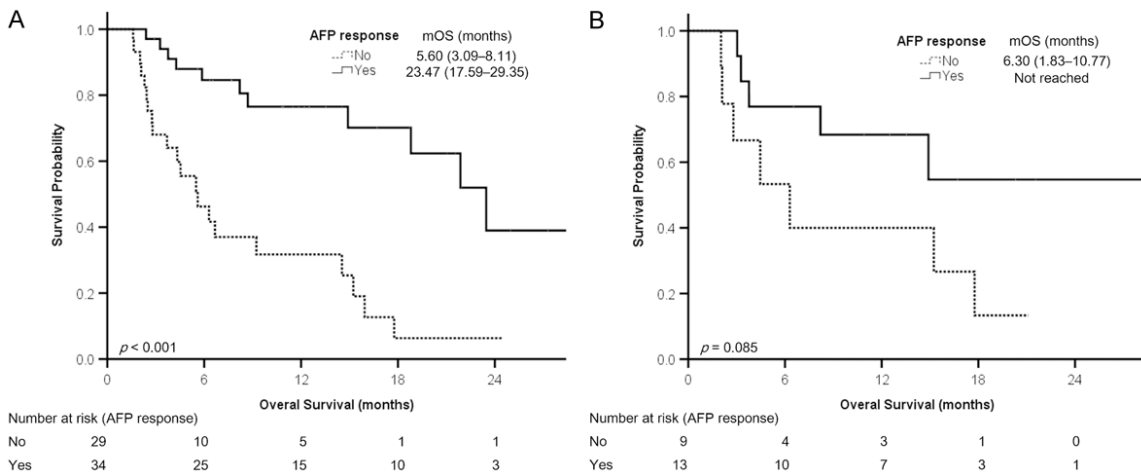
*Combination therapy includes tyrosine kinase inhibitors, radiofrequency ablation, transarterial chemoembolization, and stereotactic body radiotherapy for hepatocellular carcinoma. AFP, α -fetoprotein; ALBI, albumin-bilirubin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CR+PR+SD, complete response plus partial response plus stable disease; DM, diabetes mellitus; EHM, extrahepatic metastasis; HBV, hepatitis B virus; HCV, hepatitis C virus; TRAEs, treatment-related adverse events; MVI, macroscopic vascular invasion; NLR, neutrophil-to-lymphocyte ratio; TTV, total tumor volume.

AFP response predicts treatment outcomes of ICI combination therapies

Supplementary Table 8. Factors associated with overall survival in 95 patients with unresectable hepatocellular carcinoma (a decline of >20% in the AFP level)

Character	Univariate analysis		Multivariate analysis	
	HR (95% CI)	P value	HR (95% CI)	P value
Age (year)	0.985 (0.959-1.012)	0.266		
Sex	Male vs. female	2.416 (0.751-7.771)	0.139	
Alcohol	Yes vs. no	1.447 (0.777-2.695)	0.244	
HBV	Yes vs. no	1.134 (0.645-1.994)	0.663	
HCV	Yes vs. no	0.727 (0.363-1.455)	0.368	
DM	Yes vs. no	0.646 (0.348-1.199)	0.166	
Grade 1-2 TRAEs	Yes vs. no	0.431 (0.243-0.763)	0.004	
Grade ≥3 TRAEs	Yes vs. no	1.755 (0.894-3.446)	0.102	
TTV (cm ³)	>1000 vs. ≤1000	2.247 (1.278-3.950)	0.005	
MVI	Yes vs. no	3.803 (1.961-7.375)	<0.001	4.039 (1.647-9.904) 0.002
EHM	Yes vs. no	1.310 (0.733-2.339)	0.362	
AFP (ng/mL)	<400 vs. ≥400	3.113 (1.744-5.557)	<0.001	
AST (U/L)	>40 vs. ≤40	3.510 (1.564-7.876)	0.002	
ALT (U/L)	>40 vs. ≤40	2.163 (1.208-3.872)	0.009	
NLR	>3.0 vs. ≤3.0	3.704 (1.657-8.280)	0.001	
Child-Pugh class	B vs. A	2.492 (1.417-4.381)	0.002	
ALBI grade	2/3 vs. 1	3.499 (1.553-7.883)	0.003	
AFP decline >20%	Yes vs. no	0.377 (0.207-0.685)	0.001	0.320 (0.150-0.683) 0.003
Combination therapy*	Yes vs. no	0.441 (0.218-0.892)	0.023	0.338 (0.122-0.938) 0.037
Best response	CR+PR+SD vs. none	0.165 (0.088-0.308)	<0.001	0.432 (0.208-0.896) 0.024

*Combination therapy includes tyrosine kinase inhibitors, radiofrequency ablation, transarterial chemoembolization, and stereotactic body radiotherapy for hepatocellular carcinoma. AFP, α-fetoprotein; ALBI, albumin-bilirubin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CR+PR+SD, complete response plus partial response plus stable disease; DM, diabetes mellitus; EHM, extrahepatic metastasis; HBV, hepatitis B virus; HCV, hepatitis C virus; TRAEs, treatment-related adverse events; MVI, macroscopic vascular invasion; NLR, neutrophil-to-lymphocyte ratio; TTV, total tumor volume.



Supplementary Figure 2. Kaplan-Meier analysis of overall survival in the subgroups of patients with or without AFP response. A. A subgroup of patients receiving combined immune checkpoint inhibitor (ICI) and tyrosine kinase inhibitor therapy. B. A subgroup of patients receiving combined ICI and stereotactic body radiotherapy for hepatocellular carcinoma. Survival is presented as the median (95% confidence interval). AFP, α-fetoprotein; mOS, median overall survival.