Supplemental material

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Table S1. Fecal microbial metabolites of HCC patients in the training cohort

Characteristics	Objective response N = 20	Progressive disease N = 21	*p* value
Primary bile acids (nM)			
CA	3211.63 (478.30 – 4052.67)	1466.75 (49.99 – 3467.24)	0.083
T-CA	25.71 (8.75 – 87.07)	12.4 (4.04 – 54.46)	0.177
G-CA	56.62 (20.67 – 126.96)	25.44 (3.05 – 86.98)	0.123
CDCA	1734.66 (459.99 – 2976.28)	1371.62 (298.84 – 2393.04)	0.499
T-CDCA	40.33 (11.16 – 158.81)	34.93 (11.49 – 60.68)	0.387
G-CDCA	47.41 (15.89 – 175.22)	40.90 (10.21 – 198.35)	0.665
Secondary bile acids (nM)			
DCA	1424.18 (5.31 – 1815.73)	1307.10 (280.27 – 1927.94)	0.534
T-DCA	7.38 (1.05 – 29.97)	5.49 (1.23 – 28.61)	0.771
G-DCA	8.66 (3.99 – 30.91)	4.79 (1.80 – 19.28)	0.253
LCA	1113.60 (35.39 – 2803.71)	1619.57 (161.32 – 3077.74)	0.449
T-LCA	3.65 (1.66 – 6.07)	3.03 (0.99 – 8.31)	0.745
G-LCA	2.83 (1.30 – 5.78)	3.16 (0.93 – 7.82)	0.913
UDCA	2215.20 (998.90 – 5006.04)	966.73 (214.71 – 2010.27)	0.025
T-UDCA	7.90 (3.10 – 26.67)	2.49 (1.28 – 4.07)	0.005
G-UDCA	5.82 (2.99 – 42.98)	4.49 (1.67 – 11.43)	0.213
UCA	1638.06 (170.71 – 2507.42)	215.35 (22.38 – 674.03)	0.014
MDCA	385.83 (86.95 – 2247.38)	117.26 (31.51 – 256.72)	0.027
Short chain fatty acids (mM)			
Lactic acid	0.37 (0.18 – 1.62)	0.22 (0.11 – 0.48)	0.279
Acetic acid	12.88 (6.97 – 19.47)	11.93 (9.39 – 21.88)	0.678
Propionic acid	4.28 (2.37 – 7.09)	4.59 (3.31 – 10.27)	0.341
Isobutyric acid	0.20 (0.18 – 0.31)	0.23 (0.13 – 0.29)	0.538
Butyric acid	1.75 (1.15 – 4.36)	2.00 (1.39 – 4.33)	0.841
2-Methylbutyric acid	0.12 (0.10 – 0.21)	0.11 (0.00 – 0.15)	0.327
Isovaleric acid	0.23 (0.18 – 0.35)	0.27 (0.15 – 0.34)	1.000
Valeric acid	0.43 (0.09 – 0.56)	0.38 (0.15 – 0.54)	0.707

The values of variables are presented with median and interquartile.

Abbreviations:

CA, cholic acid; CDCA, chenodeoxycholic acid; DCA, deoxycholic acid; G, glycine conjugated species; LCA, lithocholic acid; MDCA, murideoxycholic acid (murocholic acid); mM, millimolar; nM, nanomolar; T, taurine conjugated species; UCA, ursocholic acid; UDCA, ursodeoxycholic acid.

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Table S2. Characteristics of HCC patients in the validation cohort (After March 2020)

Characteristics	Validation cohort
	N = 33
Age, y	66.3 (54.8 – 76.4)
Sex (male), n (%)	27 (81.8)
HBsAg-positive, n (%)	19 (57.6)
Anti-HCV-positive, n (%)	8 (24.2)
Max. tumor size, cm	5.9 (2.6 – 10.3)
Tumor > 50% liver volume, n (%)	4 (12.1)
Multiple tumors, n (%)	29 (87.9)
Portal vein invasion, n (%)	17 (51.5)
Extrahepatic metastasis, n (%)	16 (48.5)
AFP, ng/mL	149.0 (13.0 – 7503.0)
≥ 400 ng/mL, n (%)	13 (39.4)
BCLC stage B/C, n (%)	6/27 (18.2/81.8)
Prothrombin time, INR	1.15 (1.09 – 1.26)
Platelet count, K/cumm	200 (127 – 265)
ALT, U/L	37 (25 – 52)
AST, U/L	54 (41 – 92)
Total bilirubin, mg/dL	0.71 (00.49 – 1.12)
Albumin, g/dL	3.8 (3.5 – 4.0)
Neutrophil-lymphocyte ratio	3.76 (2.86 – 5.52)
Presence of ascites, n (%)	11 (33.3)
Child-Pugh score	6 (5 – 6)
Child-Pugh class A/B/C, n (%)	33/0/0 (100.0/0/0)
ALBI grade 1/2/3, n (%)	12/21/0 (36.4/63.6/0)
First line systemic therapy, n (%)	25 (75.8)
Prior therapy to ICI, n (%)	
Surgical resection	12 (36.4)
RFA	11 (33.3)
TACE	18 (54.5)
Sorafenib	8 (24.2)
Nivolumab/Pembrolizumab, n (%)	7/26 (21.2/78.8)
Combined ICI with TKI, n (%)	28 (84.8)
Immune-related AEs	7 (21.2)

The values of continuous variables are presented with median and interquartile range

Abbreviations:
ALBI grade, albumin-bilirubin grade; AEs, adverse events; AFP, alpha fetoprotein; AL(S)T, alanine(aspartate) aminotransferase; BCLC stage, Barcelona-Clinic liver cancer stage; HBsAg, hepatitis B virus surface antigen; HCV, hepatitis C; ICI, immune checkpoint inhibitors; INR, international normalized ratio; NLR, neutrophil-lymphocyte ratio; RFA, radiofrequency ablation; TACE, trans-arterial chemoembolization; TKI, tyrosine kinase inhibitor.

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Table S3. Tumor response associated with gut microbial composition in validation group

	Prevotella 9		*Lachnoclostridium*		*Lachnoclostridium-Prevotella 9*[#]		
	Enriched	Depleted	Depleted	Enriched	Poor	Fair	Good
	n = 11	n = 22	n = 7	n = 26	n = 4	n = 10	n = 19
PR	2 (18.2)	10 (45.5)	0	12 (46.2)	0 (0)	2 (20.0)	10 (52.6)
SD	5 (45.5)	10 (45.5)	2 (28.6)	13 (50.0)	0 (0)	7 (70.0)	8 (42.1)
PD	4 (36.4)	2 (9.1)	5 (71.4)	1 (3.8)	4 (100.0)	1 (10.0)	1 (5.3)
ORR	2 (18.2)	10 (45.5)	0 (0)	12 (46.2)	0 (0)	2 (20.0)	10 (52.6)
p value	0.249		0.032		0.060		
DCR	7 (63.6)	20 (90.9)	2 (28.6)	25 (96.2)	0 (0)	9 (90.0)	18 (94.7)
p value	0.149		< 0.001		< 0.001		

[#]Signature of fecal microbiota

Good signature: coexistence of depleted *Prevotella 9* and enriched *Lachnoclostridium*

Poor signature: coexistence of enriched *Prevotella 9* and depleted *Lachnoclostridium*

Fair signature: coexistence of depleted both two bacteria or enriched both two bacteria

Abbreviations:

DCR, disease control rate; ORR, objective response rate; PD, progressive disease; PR, partial response; SD, stable disease.