Baseline Characteristics of Patients With Hepatocellular Carcinoma by Bone Metastasis Status

Parameters	Total/Number (84)	Bone metastasis		P-values	SMD
		No (45)	Yes (39)		
Age	53.20 ± 10.79	52.09 ± 8.89	54.49 ± 12.63	0.325	0.220
Sex				0.936	0.032
Female	33 (39.3%)	17 (37.8%)	16 (41.0%)		
Male	51 (60.7%)	28 (62.2%)	23 (59.0%)		
Pathological type of primary site				0.650*	0.044
Well differentiated HCC	2 (2.4%)	2 (4.4%)	0 (0.0%)		
Moderately differentiated HCC	42 (50.0%)	23 (51.1%)	19 (48.7%)		
Poorly differentiated HCC	37 (44.0%)	19 (42.2%)	18 (46.2%)		
Cholangiocarcinoma	3 (3.6%)	1 (2.2%)	2 (5.1%)		
Para-tumor tissue available				-	-
No	39 (46.4%)	0 (0.0%)	39 (100.0%)		
Yes	45 (53.6%)	45 (100.0%)	0 (0.0%)		
Primary tumor tissue available				-	-
No	10 (11.9%)	0 (0.0%)	10 (25.6%)		
Yes	74 (88.1%)	45 (100.0%)	29 (74.4%)		
Bone lesion tissue available				-	-
No	63 (75.0%)	45 (100.0%)	18 (46.2%)		
Yes	21 (25.0%)	0 (0.0%)	21 (53.8%)		
Lung metastasis				0.218	0.157
No	48 (57.1%)	29 (64.4%)	19 (48.7%)		
Yes	36 (42.9%)	16 (35.6%)	20 (51.3%)		
Lymph node metastasis				0.365	0.115
No	59 (70.2%)	34 (75.6%)	25 (64.1%)		
Yes	25 (29.8%)	11 (24.4%)	14 (35.9%)		
Adrenal metastasis				0.659*	0.032
No	79 (94.0%)	43 (95.6%)	36 (92.3%)		
Yes	5 (6.0%)	2 (4.4%)	3 (7.7%)		
Brain metastasis				1.000*	0.022
No	83 (98.8%)	44 (97.8%)	39 (100.0%)		
Yes	1 (1.2%)	1 (2.2%)	0 (0.0%)		
tRF expression	3.29 (2.48, 4.55)	2.99 (2.31, 3.56)	4.34 (2.60, 5.98)	0.001	0.917
ALKBH5 expression	5.73 ± 1.92	5.07 ± 1.52	6.51 ± 2.06	<0.001	0.793
INPP4B expression	3.50 (2.35, 5.42)	5.29 (4.19, 6.44)	2.25 (1.29, 3.10)	<0.001	-2.130

Footnotes. Continuous variables are presented as mean ± SD when normally distributed (Shapiro-Wilk P > 0.05) and compared with the t test, or as median (IQR) and compared with the Wilcoxon rank-sum test. Categorical variables are presented as No. (%) and compared with the chi-square test, or the Fisher exact test when any expected cell count was below 5 (marked with an asterisk). Specimen availability rows are descriptive only and were not tested, because the difference between groups is determined by study design. |SMD| > 0.10 was considered to indicate imbalance.

Abbreviations. BM, bone metastasis; HCC, hepatocellular carcinoma; IQR, interquartile range; SD, standard deviation; SMD, standardized mean difference; tRF, transfer RNA-derived fragment.

Baseline Characteristics of Patients With Hepatocellular Carcinoma by tRF Expression Levele

Parameters	Total/Number (84)	tRF expression		P-values	SMD
		Low (42)	High (42)		
Bone metastasis status				0.016	0.263
No	45 (53.6%)	28 (66.7%)	17 (40.5%)		
Yes	39 (46.4%)	14 (33.3%)	25 (59.5%)		
Age	53.20 ± 10.79	54.69 ± 9.49	51.71 ± 11.88	0.208	-0.277
Sex				0.503	0.073
Female	33 (39.3%)	18 (42.9%)	15 (35.7%)		
Male	51 (60.7%)	24 (57.1%)	27 (64.3%)		
Pathological type of primary site				0.936*	0.074
Well differentiated HCC	2 (2.4%)	1 (2.4%)	1 (2.4%)		
Moderately differentiated HCC	42 (50.0%)	22 (52.4%)	20 (47.6%)		
Poorly differentiated HCC	37 (44.0%)	18 (42.9%)	19 (45.2%)		
Cholangiocarcinoma	3 (3.6%)	1 (2.4%)	2 (4.8%)		
Para-tumor tissue available				-	-
No	39 (46.4%)	14 (33.3%)	25 (59.5%)		
Yes	45 (53.6%)	28 (66.7%)	17 (40.5%)		
Primary tumor tissue available				-	-
No	10 (11.9%)	1 (2.4%)	9 (21.4%)		
Yes	74 (88.1%)	41 (97.6%)	33 (78.6%)		
Bone lesion tissue available				-	-
No	63 (75.0%)	36 (85.7%)	27 (64.3%)		
Yes	21 (25.0%)	6 (14.3%)	15 (35.7%)		
Lung metastasis				0.659	0.048
No	48 (57.1%)	23 (54.8%)	25 (59.5%)		
Yes	36 (42.9%)	19 (45.2%)	17 (40.5%)		
Lymph node metastasis				0.811	0.026
No	59 (70.2%)	30 (71.4%)	29 (69.0%)		
Yes	25 (29.8%)	12 (28.6%)	13 (31.0%)		
Adrenal metastasis				1.000*	0.05
No	79 (94.0%)	39 (92.9%)	40 (95.2%)		
Yes	5 (6.0%)	3 (7.1%)	2 (4.8%)		
Brain metastasis				1.000*	0.11
No	83 (98.8%)	42 (100.0%)	41 (97.6%)		
Yes	1 (1.2%)	0 (0.0%)	1 (2.4%)		
ALKBH5 expression	5.73 ± 1.92	5.21 ± 1.57	6.26 ± 2.12	0.012	0.562
INPP4B expression	3.50 (2.35, 5.42)	4.32 (3.13, 5.81)	2.92 (1.64, 4.97)	0.006	-0.571

Footnotes. Patients were dichotomized at the cohort-wide median of tRF expression (3.285). Continuous variables are presented as mean ± SD or median (IQR) according to the Shapiro-Wilk test and compared with the t test or Wilcoxon rank-sum test; categorical variables are presented as No. (%) and compared with the chi-square test or the Fisher exact test (marked with *). Specimen availability rows are descriptive only.

Abbreviations. BM, bone metastasis; IQR, interquartile range; SD, standard deviation; SMD, standardized mean difference; tRF, transfer RNA-derived fragment.

Univariable and Multivariable Cox Proportional Hazards Analyses for Overall Survival

	Univariable analysis		Multivariable analysis Model 1		Multivariable analysis Model 2		Multivariable analysis Model 3	
Characteristics	**HR (95% CI)**	**P-value**	**HR (95% CI)**	**P-value**	**HR (95% CI)**	**P-value**	**HR (95% CI)**	**P-value**
Age, y	0.987 (0.963-1.012)	0.307						
Sex								
Female	1.000 (reference)							
Male	1.073 (0.613-1.878)	0.805						
Pathological differentiation								
Non-poorly differentiated	1.000 (reference)		1.000 (reference)		1.000 (reference)		1.000 (reference)	
Poorly differentiated	1.836 (1.069-3.151)	0.028	1.969 (1.132-3.424)	0.016	2.040 (1.174-3.545)	0.011	2.110 (1.213-3.671)	0.008
Specimen used for tRF assay								
Primary tumor	1.000 (reference)							
Bone lesion	1.837 (0.818-4.127)	0.141						
Lung metastasis								
No	1.000 (reference)							
Yes	1.493 (0.862-2.587)	0.153						
Lymph node metastasis								
No	1.000 (reference)		1.000 (reference)		1.000 (reference)		1.000 (reference)	
Yes	2.434 (1.338-4.426)	0.004	2.580 (1.388-4.796)	0.003	2.127 (1.124-4.028)	0.020	2.530 (1.356-4.720)	0.004
Adrenal metastasis								
No	1.000 (reference)							
Yes	1.973 (0.706-5.514)	0.195						
Bone metastasis								
No	1.000 (reference)							
Yes	1.132 (0.656-1.956)	0.655						
tRF expression	1.182 (0.988-1.414)	0.067	1.124 (0.944-1.339)	0.190				
ALKBH5 expression	1.309 (1.121-1.529)	<0.001			1.253 (1.070-1.469)	0.005		
INPP4B expression	0.868 (0.740-1.018)	0.082					0.888 (0.759-1.038)	0.136

Footnotes. Three multivariable models were fitted, each combining the clinical variables with $P < .05$ in univariable analysis (pathological differentiation and lymph node metastasis) with one gene of interest: model 1 with tRF, model 2 with ALKBH5, and model 3 with INPP4B. The three genes were not entered into a single model. Events per variable was calculated as the number of deaths divided by the number of model coefficients (54/3 = 18.0 for each model), and discrimination as the C index (model 1, 0.680; model 2, 0.706; model 3, 0.666). The proportional hazards assumption was assessed with scaled Schoenfeld residuals and was satisfied in all models (all $P \geq 0.05$; smallest P = 0.109). Overall survival was measured from the date of primary tumor

diagnosis. For categorical variables the largest category is used as the reference. Binary variables with fewer than 5 patients in either level were not modeled.

Abbreviations. CI, confidence interval; HR, hazard ratio; tRF, transfer RNA-derived fragment.