

ORIGINAL RESEARCH

Ghelfi et al

PD1 and TIM3 expression is associated with very early hepatocellular carcinoma recurrence after percutaneous thermal ablation

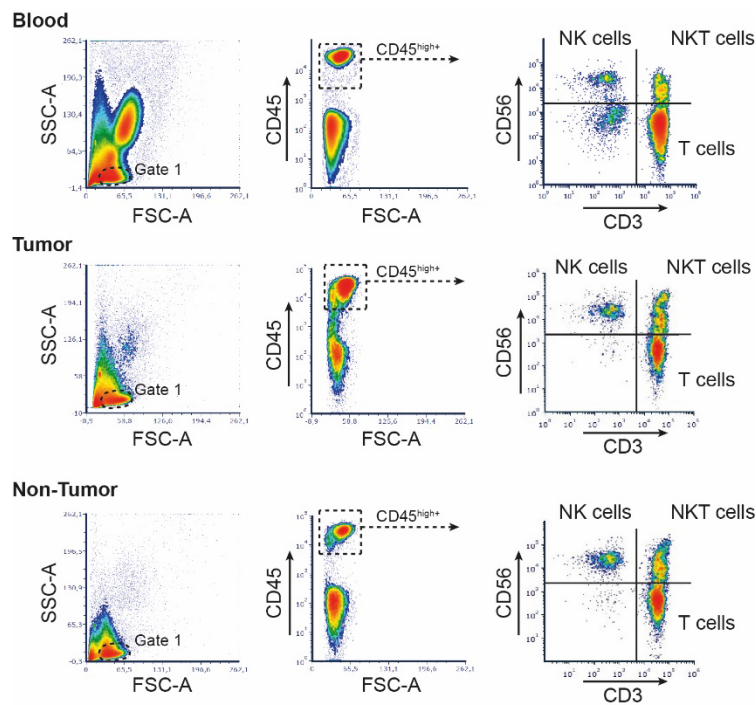


Figure S1 Gating strategy for flow cytometry analysis.

Fresh samples of peripheral blood, nontumoral and intratumoral liver tissue were stained for FACS analyses. After exclusion of dead cells and doublets, lymphocytes were gated based on SSC and FSC and then based on CD45 expression. We identified CD3⁺CD56⁺ NK cells, CD3⁺CD56⁺ NKT cells and CD3⁺CD56⁻ classical T cells as previously described ¹.

Reference:

1. Macek Jilkova Z, Aspod C, Kurma K, et al. Immunologic Features of Patients With Advanced Hepatocellular Carcinoma Before and During Sorafenib or Anti-programmed Death-1/Programmed Death-L1 Treatment. *Clin Transl Gastroenterol*. Jul 2019;10(7):e00058. doi:10.14309/ctg.0000000000000058

Table S1 The baseline frequency of immune cells in peripheral blood, nontumoral and intratumoral liver tissue

	Prolonged recurrence-free group (n=22)		Very early recurrence group (n=9)		P value
Blood					
% of CD45 ⁺ cells, <i>median [IQR]</i>					
T cells	63.2	[56.8-69.0]	62.5	[56.8-71.3]	ns
CD4 ⁺ T cells	46.4	[39.8-49.7]	46.0	[43.2-51.1]	ns
CD8 ⁺ T cells	16.7	[10.8-21.4]	19.2	[11.5-21.2]	ns
NKT cells	1.8	[1.1-6.6]	5.2	[1.6-13.0]	ns
NK cells	13.1	[7.5-18.6]	10.0	[5.3-15.7]	ns
% of T cells, median [IQR]					
CD4 ⁺ T cells	76.4	[62.6-81.4]	70.7	[69.4-79.5]	ns
CD8 ⁺ T cells	23.7	[18.6-37.4]	29.3	[20.5-30.6]	ns
Non-tumoral					
weight of biopsy sample in mg, median [IQR]	8.6	[7.4-9.2]	8.6	[5.5-8.6]	ns
% of CD45 ⁺ cells, <i>median [IQR]</i>					
T cells	44.3	[36.0-49.0]	38.4	[31.0-50.0]	ns
CD4 ⁺ T cells	19.7	[17.2-23.2]	20.9	[15.6-24.6]	ns
CD8 ⁺ T cells	22.8	[17.5-26.4]	17.7	[14.8-21.8]	ns
NKT cells	14.7	[8.1-22.5]	15.6	[7.4-29.9]	ns
NK cells	23.0	[21.3-34.6]	31.5	[25.0-32.7]	ns
% of T cells, median [IQR]					
CD4 ⁺ T cells	49.4	[41.0-56.9]	55.6	[49.3-59.5]	ns
CD8 ⁺ T cells	50.6	[43.1-59.0]	44.4	[40.5-50.7]	ns
Intra-tumoral					
weight of biopsy sample in mg, median [IQR]	7.4	[5.0-7.4]	7.4	[6.8-7.4]	ns
% of CD45 ⁺ cells, <i>median [IQR]</i>					
T cells	52.0	[44.9-65.5]	53.9	[47.6-56.5]	ns
CD4 ⁺ T cells	33.0	[26.6-41.1]	35.6	[28.8-43.2]	ns
CD8 ⁺ T cells	18.9	[13.0-21.9]	17.7	[13.8-20.6]	ns
NKT cells	15.4	[7.9-17.6]	15.7	[6.6-24.2]	ns
NK cells	15.9	[11.3-20.4]	21.3	[14.5-23.6]	ns
% of T cells, median [IQR]					
CD4 ⁺ T cells	63.7	[55.0-75.3]	64.3	[59.7-76.2]	ns
CD8 ⁺ T cells	34.3	[24.8-43.6]	36.7	[23.8-40.3]	ns

n/a: not applicable; ns: not significant; IQR: Interquartile range;

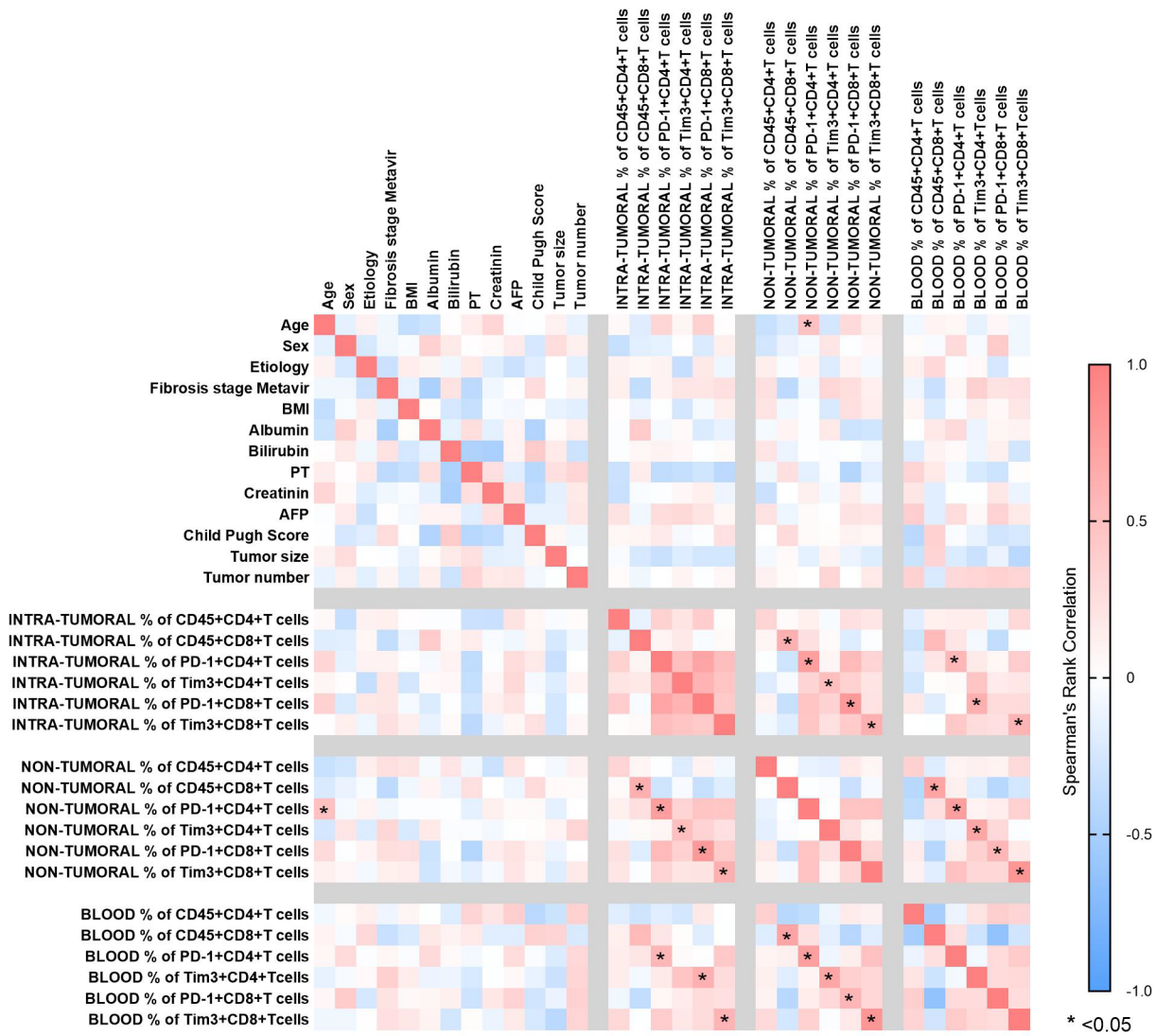


Figure S2 Correlation matrix of HCC patient clinical characteristics and immune signature at baseline

Heatmap of correlation of patient characteristics and frequencies of intra-tumoral, non-tumoral and blood T cells from HCC patients at the baseline before the ablation procedure. Color corresponds to Spearman's rank correlation coefficient r , $n = 31$. * $p < 0.05$ represent statistically significant correlations after the adjustment.