

Flow cytometry staining and analysis

- (I) Marked flow pipe, 100 μ L blood samples (50 μ L in absolute count tube) were added into each tube, and then fluorescein labeled cell membrane molecular antibodies were added according to the antibody instructions (*Table S1*), and incubated at 4 °C in dark for 20–30 minutes.
- (II) After the staining, 1mL of red blood cell lysate was added into each tube (450ul in the absolute counter tube). After the oscillation, it was left for 10 minutes at room temperature and in dark, and then centrifuged at 300g for 5 minutes (the absolute counter tube was directly put on the machine without centrifugation).
- (III) The cells were resuspended with PBS and centrifuged at 300g for 5 min. The cells were washed twice and added with 200 L PBS and prepared for loading.
- Add an appropriate Staining Buffer. Adjust cell concentration to 106/100 L/Test;
 - Labeled flow tubes were 1, 2, 3, 4, and 5 (*Table S2*). Each tube was labeled as shown in the table below. Fluorescein labeled cell membrane molecular antibodies were added according to the antibody instructions, and incubated at 4 °C in dark for 20–30 minutes.
 - After the staining, the cells were resuspended with PBS (300 percP-CY5.5) for 5 minutes, and then washed twice. Cells were resuspended with 200 L PBS (10 L 7AAD (percP-CY5.5 channel detection) active dye was added into each tube.

Table S1 Flow cytometry antibody labeling details

Anti-bodies	Companies	Product No.
Tregs		
CD4-PE	Biolegend	317410
CD25-PE	Biolegend	302606
CD127-APC	Biolegend	351316
CD3-4-8, PD-1		
CD3-PE-cy7	Biolegend	344816
CD4-PE	Biolegend	317410
CD8-FITC	Biolegend	301050
PD-1-APC	Biolegend	329908
CD45-PERCP-CY5.5	BD	340953
NK,B absolute count		
CD3-FITC	Biolegend	300306
CD56-PE	Biolegend	362508
CD16-PE	Biolegend	360704
CD19-APC	Biolegend	302212
CD45-PERCP-CY5.5	BD	340953
Absolute counter tube	BD	340334

Table S2 Flow cytometry antibody labeling details

	FITC	PE	APC	PE-cy7	APC-cy7
1*	CD4		IgG1-APC	CD3	CD8
2	CD4		PD-L1	CD3	CD8
3*	CD103	IgG1	PD-1	CD3	CD8
4*	CD103	TIM-3	IgG1	CD3	CD8
5	CD103	TIM-3	PD-1	CD3	CD8

1*, PD-L1 for continuous expression, the tube was set to a fluorescence minus one control (FMO) tube with no clustering; 3*, 4*, Tim-3 and PD-1, the cluster was not obvious in individual tumor tissues, and the two tubes were set as FMO tubes with no. 5 tubes.

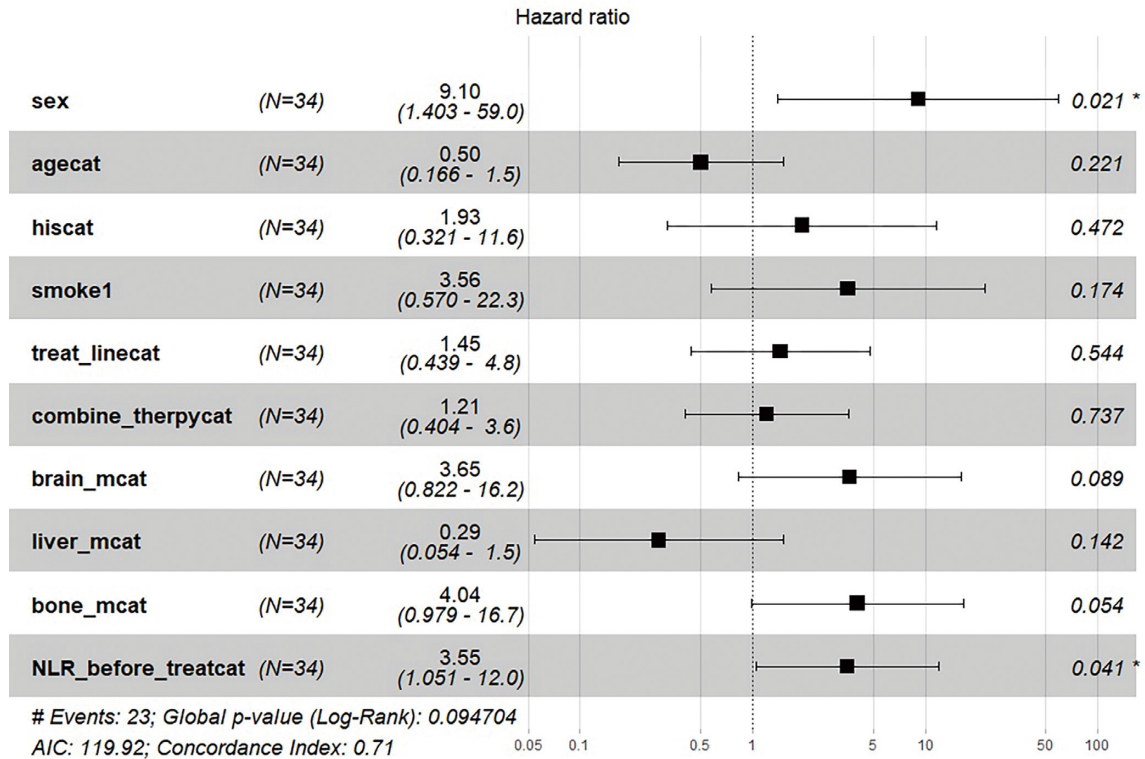


Figure S1 Forest plot for the associations between the 34 participant characteristics and PFS by Cox proportional hazards regression models. sex = gender; agecat = age category; smoke1 = smoker; treat_linecat = treat line; combine_therpycat = treatment model; brain_mcat = brain metastasis; liver_mcat = liver metastasis; bone_mcat = bone metastasis; NLR_before_treatcat = NLR.