

Appendix 1 Supplemental methods

Transarterial chemoembolization (TACE) procedure

Under either local or general anesthesia, multiple angiographic steps were performed using a 5-F catheter via femoral artery access to identify the tumor-feeding arteries. After catheterizing the selected vessel with a 2.4-F microcatheter, chemoembolization was performed. An emulsion was prepared by mixing 10 mL of Lipiodol with 10 mL of chemotherapeutic agent (50 mg epirubicin), using a push-and-pull method to create a homogeneous and stable solution. The volume of the emulsion was determined based on the patient's physical performance and tumor size. Following this, 100–300- μm gelatin sponge particles were injected to achieve the embolization endpoint. For drug-eluting bead (DEB)TACE, HepaSphere Microspheres (Merit Medical Systems, USA), sized 30–60 μm , were loaded with 50 mg of Epirubicin per vial, which was dissolved in saline. The drug-loaded HepaSphere was then mixed with 20 mL of non-ionic contrast agent to form a uniform suspension, which was administered intra-arterially. Additional non-resorbable bland microparticles were added when needed. The technical end point of this procedure was considered to be achieved when the entire dose of chemotherapy was administered, whereas the angiographic end point was determined to be when the arterial flow was reduced to the point that it took 2–5 beats to clear the contrast column.

Construction process, parameter estimation methods, and model fine-tuning

The joint model (JM) was constructed following a structured, two-stage process. First, for the longitudinal part, the linear mixed model was fitted to capture individual variation in trajectory over time at patient-specific level. Linear mixed model allows inherently irregular measurement and varying follow-up times across patients. Second, the estimated individual Tregs trajectory as time-varying covariate was incorporated into the Cox proportional-hazards model (time-to-event analysis). To ensure the model fitting, all relevant time-varying covariates and their possible interaction terms were initially as fixed effects. Model simplification was then performed in a step-wise way. Non-significant interaction terms were removed first. Then, the random effects was refined by sequentially removing non-significant random effects based on Bayesian Information Criterion.

Parameters for the joint model were estimated simultaneously using a full maximum likelihood approach. The proportional hazards assumption for the Cox component was verified using Schoenfeld residual plots, with log-transformation applied if necessary. The unstandardized coefficients of the JMs were used to quantify the association between Tregs changes over time and the risk of endpoint. The unstandardized coefficient was used for calculating hazard ratio and its and 95% confidence intervals. Model validation and comparison were conducted to ensure accuracy and avoid overfitting. Nested models were compared using the Bayesian Information Criterion and likelihood ratio tests, confirming that the final model was both statistically supported and clinically interpretable.

Appendix 2 Core code in R

```
install.packages("JM")
install.packages("JMbayes2")
install.packages("tidyverse")
library(JMbayes2)
library(JM)
library(tidyverse)
# cox model
# fixed variable
CoxFit <- coxph(Surv(PFS, PD) ~ 1, data = coxdata) # null model
CoxFit_BCLC <- coxph(Surv(PFS, PD) ~ BCLC, data = coxdata)
CoxFit_Sex <- coxph(Surv(PFS, PD) ~ Sex, data = coxdata)
CoxFit_Embolization <- coxph(Surv(PFS, PD) ~ Embolization, data = coxdata)
CoxFit_Decompensation <- coxph(Surv(PFS, PD) ~ Decompensation, data = coxdata)
```

```

# time-dependent variable
Fm_Tregs <- lme(Tregs~ times ,data = jointdata, random = ~ times | ID)
Fm_CPscore <- lme(CPscore ~ times ,data = jointdata, random = ~ times | ID)
Fm_AFP <- lme(log(AFP) ~ times ,data = jointdata, random = ~ times | ID)

```

univariate joint model

```

jointFit_BCLC <- jm(CoxFi_BCLC, list(Fm_Tregs), time_var = "times",
  n_iter = 12000L, n_burnin = 2000L, n_thin = 5L)
jointFit_Sex <- jm(CoxFi_Sex, list(Fm_Tregs), time_var = "times",
  n_iter = 12000L, n_burnin = 2000L, n_thin = 5L)
jointFit_Embolization <- jm(CoxFi_Embolization, list(Fm_Tregs), time_var = "times",
  n_iter = 12000L, n_burnin = 2000L, n_thin = 5L)
jointFit_Decomensation <- jm(CoxFi_Decomensation, list(Fm_Tregs), time_var = "times",
  n_iter = 12000L, n_burnin = 2000L, n_thin = 5L)

jointFit_CPscore <- jm(CoxFit, list(Fm_Tregs, Fm_CPscore), time_var = "times",
  n_iter = 12000L, n_burnin = 2000L, n_thin = 5L)
jointFit_AFP <- jm(CoxFit, list(Fm_Tregs, Fm_CPscore), time_var = "times",
  n_iter = 12000L, n_burnin = 2000L, n_thin = 5L)

summary(jointFit)

```

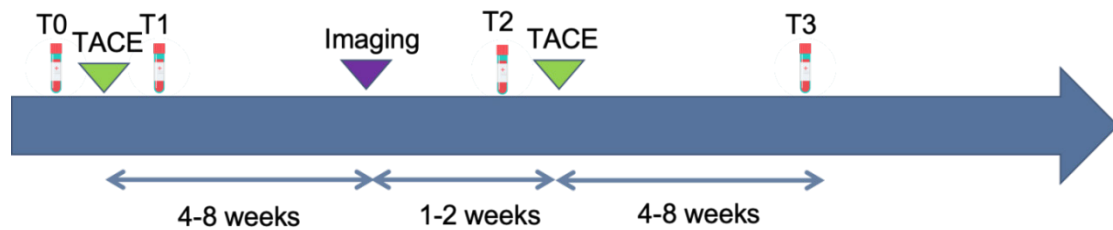


Figure S1 Blood collection procedure.

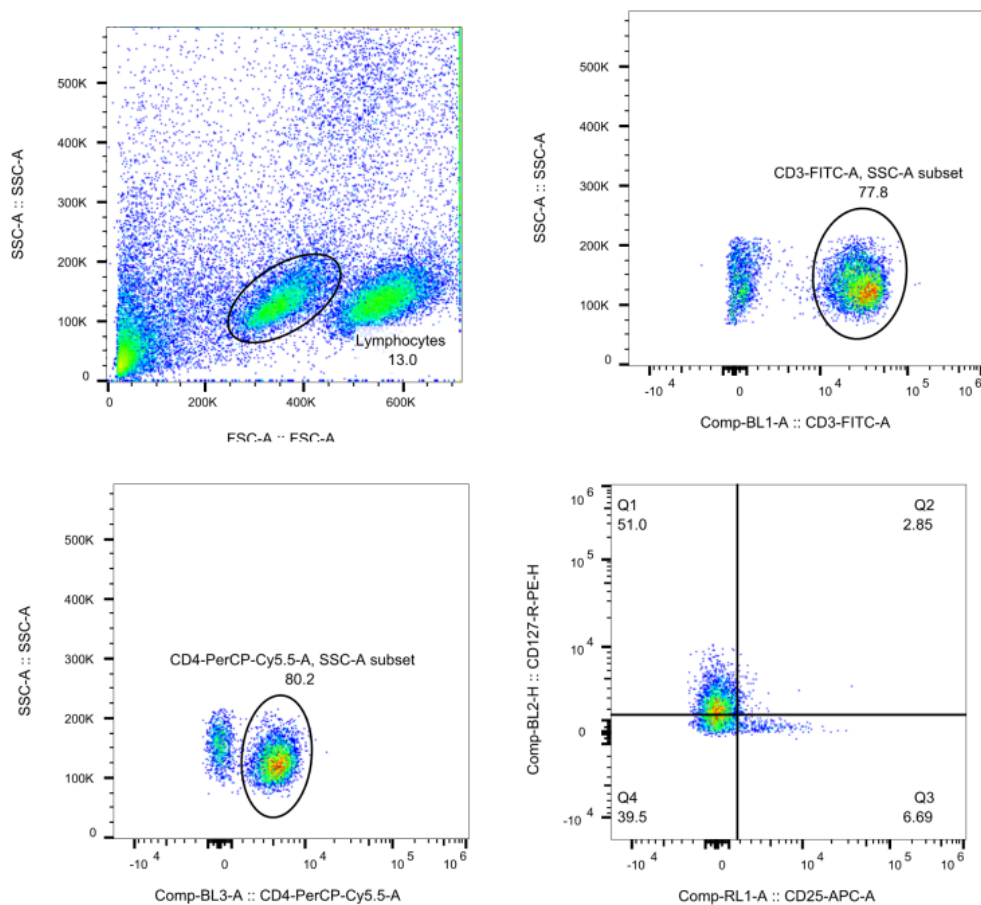


Figure S2 Gate strategy and representative flow dots of CD4+CD25+CD127dim/- Tregs.

Table S1 Time-varying variables during treatment

Characteristic	Baseline(N=30)	T1(N=19)	T2(N=30)	T3(N=30)
Child-Pugh score	6.00 (5.00-6.00)	6.00 (5.00-7.00)	6.00 (5.00-7.00)	7.00 (5.25-8.00)
Child-Pugh class				
A	23 (76.67%)	13 (54.17%)	20 (66.67%)	13 (37.14%)
B	7 (23.33%)	6 (25.00%)	10 (33.33%)	15 (42.86%)
C	-	-		2 (5.71%)
Tregs	6.96 (5.28-10.37)	6.81±2.29	7.06±2.52	5.84 (4.97-8.40)
AFP	38.12 (4.93-538.22)	24.25 (5.99-292.48)	18.44 (3.50-168.58)	12.55 (6.12-168.75)
CD3+ T cells	72.27 (66.19-77.43)	75.58 (71.79-77.98)	73.50±7.48	73.01±8.32
CD4+ T cells	40.31±8.54	40.41±8.66	42.05 (39.05-44.63)	41.46±6.81
CD8+ T cells	28.65±7.11	30.54±7.62	30.52±7.81	26.50 (25.07-36.05)
Ratio of CD4+ and CD8+ T cells	1.52±0.55	1.16 (0.93-1.82)	1.47±0.53	1.51±0.45
CD16+CD56+ T cells	11.85 (8.08-18.96)	13.52 (9.23-18.50)	12.44 (8.79-16.34)	14.09±7.70
CD19+ T cells	14.24±6.43	10.97±4.87	12.40±6.32	12.20±5.48

Table S2 Test of interaction term of sex and times on Tregs trajectory

effect	group	term	estimate	std.error	statistic	df	p.value
fixed		(Intercept)	7.264	0.952	7.631	28.872	0.000
fixed		Sex: male	0.714	1.111	0.642	28.858	0.526
fixed		times	-0.097	0.268	-0.362	30.289	0.720
fixed		Sex: male:times	-0.321	0.313	-1.025	30.278	0.313
ran_pars	ID	sd__(Intercept)	2.301				
ran_pars	ID	cor__(Intercept).times	-0.278				
ran_pars	ID	sd__times	0.247				
ran_pars	Residual	sd__Observation	1.582				

Table S3 Test of interaction term of BCLC and times on Tregs trajectory

effect	group	term	estimate	std.error	statistic	df	p.value
fixed		(Intercept)	8.768	0.750	11.697	29.094	0.000
fixed		BCLC: B	-1.646	0.969	-1.700	29.199	0.100
fixed		times	-0.657	0.208	-3.158	29.914	0.004
fixed		BCLC: B:times	0.544	0.269	2.025	30.001	0.052
ran_pars	ID	sd__(Intercept)	2.195				
ran_pars	ID	cor__(Intercept).times	0.028				
ran_pars	ID	sd__times	0.097				
ran_pars	Residual	sd__Observation	1.580				

Table S4 Test of interaction term of embolization technique and times on Tregs trajectory

effect	group	term	estimate	std.error	statistic	df	p.value
fixed		(Intercept)	8.060	0.674	11.957	28.924	0.000
fixed		Embolization: D-TACE	-0.582	0.986	-0.590	28.881	0.560
fixed		times	-0.371	0.192	-1.930	30.053	0.043
fixed		Embolization D-TACE:times	0.082	0.281	0.291	30.019	0.773
ran_pars	ID	sd__(Intercept)	2.305				
ran_pars	ID	cor__(Intercept).times	-0.291				
ran_pars	ID	sd__times	0.277				
ran_pars	Residual	sd__Observation	1.583				

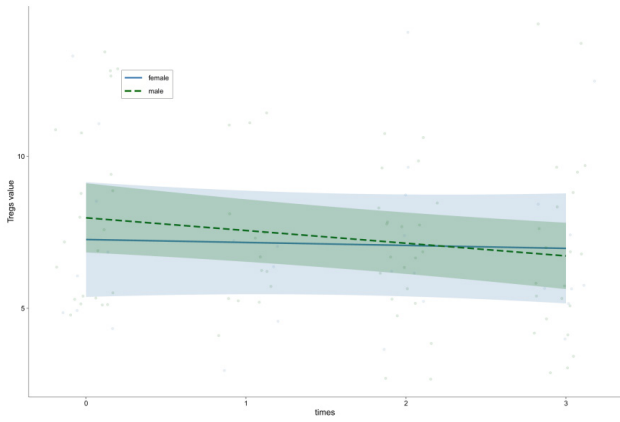


Figure S3 Test of interaction term of sex and times on Tregs trajectory. No significant difference in the trajectories of Tregs between male and female as observed.

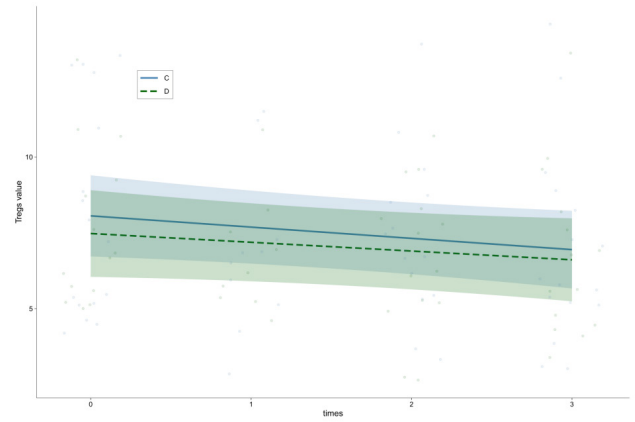


Figure S5 Test of interaction term of embolization technique and times on Tregs trajectory. No significant difference in the trajectories of Tregs between conventional TACE and drug-eluting TACE was observed.

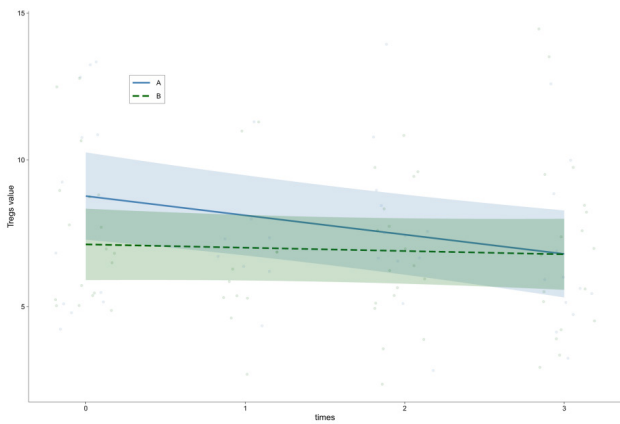


Figure S4 Test of interaction term of BCLC and times on Tregs trajectory. No significant difference in the trajectories of Tregs between BCLC A and B was observed.

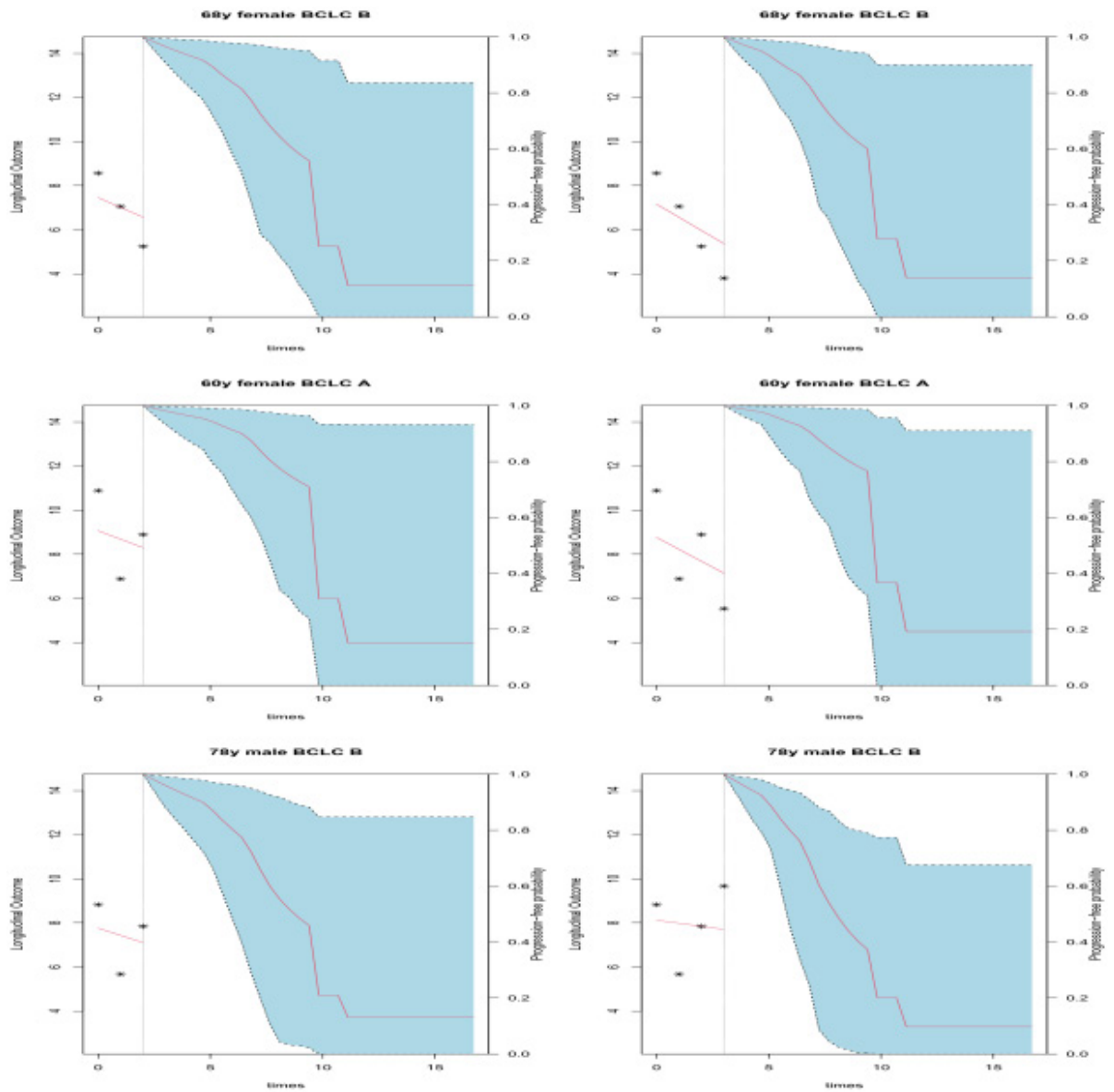


Figure S6 Additional representative cases presenting specific association of Tregs trajectories on tumor progression risk prediction. Each line was the same patient. The left side is the curve of progression-free probability after three Tregs measurements