Table S3. Patients baseline characteristics before and after propensity score matching

Characteristics	Before Matching			After Matching (1:1)		
	ACT No. (%) (n=816)	No ACT No. (%) (n=212)	P-value	ACT No. (%) (n=185)	No ACT No. (%) (n=185)	P-value
Sex			0.20			0.92
Male	490 (60.05%)	117 (55.19%)		108 (58.4)	106 (57.3)	
Female	326 (39.95%)	95 (44.81%)		77 (41.6)	79 (42.7)	
Age, mean ± SD, years	57.10 ± 12.32	59.72 ± 12.41	0.006	59.14 ± 12.80	59.49 ± 12.23	0.79
BMI, mean ± SD	22.59 ± 3.18	22.59 ± 3.52	0.98	22.67 ± 3.80	22.62 ± 3.50	0.90
T stage			<0.001			0.66
T1	15 (1.84%)	32 (15.09%)		14 (7.6)	12 (6.5)	
T2	93 (11.40%)	79 (37.26%)		70 (37.8)	75 (40.5)	
T3	647 (79.29%)	91 (42.92%)		95 (51.4)	88 (47.6)	
T4	61 (7.48%)	10 (4.72%)		6 (3.2)	10 (5.4)	
N stage			<0.001			0.45
N0	406 (49.75%)	160 (75.47%)		125 (67.6)	133 (71.9)	
N1	276 (33.82%)	37 (17.45%)		47 (25.4)	37 (20)	
N2	134 (16.42%)	15 (7.08%)		13 (7)	15 (8.1)	
Tumor differentiation			<0.001			0.55
Well-moderate	492 (60.29%)	154 (72.64%)		133 (71.9)	135 (73)	
Poor-undifferentiated	280 (34.31%)	39 (18.40%)		43 (23.2)	37 (20)	
Unknown	44 (5.39%)	19 (8.96%)		9 (4.9)	13 (7)	
Mucinous type (Histological type)			0.71			
Yes	23 (2.82%)	5 (2.36%)		5 (2.7)	5 (2.7)	
No	793 (97.18%)	207 (97.64%)		180 (97.3)	180 (97.3)	

Abbreviation: ACT, adjuvant chemotherapy; BMI, body mass index; SD, standard deviation.

Table S2. Baseline characteristics of patients in the training and validation sets

Clinicopathologic Feature	Training set		Validation set		P-value
	No.	%	No.	%	
Sex					0.559
Male	542	59.36	65	56.52	
Female	371	40.64	50	43.48	
Age, years					0.010
<65	633	69.33	66	57.39	
≥65	280	30.67	49	42.61	
BMI					0.461
<24	620	67.91	82	71.30	
≥24	293	32.09	33	28.70	
Surgical approach					0.318
OR	705	77.22	84	73.04	
LR	208	22.78	31	26.96	
Primary site					0.232
Colon	443	48.52	49	42.61	
Rectal	470	51.48	66	57.39	
Tumor differentiation					<0.001
Well	46	5.04	55	47.83	
Moderate	499	54.65	46	40.00	
Poor- undifferentiated	310	33.95	9	7.83	
Unknown	58	6.35	5	4.35	
Histological type					0.127
Adenocarcinomas	891	97.59	109	94.78	
Mucinous adenocarcinomas	18	1.97	4	3.48	
Signet-ring cell carcinomas	4	0.44	2	1.74	
T stage					<0.001
T1	42	4.60	5	4.35	
T2	152	16.65	20	17.39	
T3	683	74.81	55	47.83	
T4	36	3.94	35	30.43	
N stage					0.866
N0	500	54.76	66	57.39	
N1	280	30.67	33	28.70	
N2	133	14.57	16	13.91	
AJCC 7^{th} ed. stage					0.687
Stage I	156	17.09	20	17.39	
Stage II	347	38.01	48	41.74	
Stage III	410	44.91	47	40.87	
Tumor deposit					0.016
Yes	841	92.11	113	98.26	
No	72	7.89	2	1.74	
Postoperative CD4+ T cells percentage					0.923
Normal	472	51.70	60	52.17	
Abnormal	441	48.30	55	47.83	
Postoperative CD4+ T cells count					0.845
Normal	830	90.91	103	90.35	
Abnormal	83	9.09	11	9.65	
Adjuvant chemotherapy					0.675
Yes	723	79.19	93	80.87	
No	190	20.81	22	19.13	

Note: BMI, body mass index; LR, laparoscopic resection; OR, open resection.

Supplementary methods:

Flow cytometry of CD4+ T cell subsets

1. Main instruments and reagents The test instruments are Beckman Coulter DxFLEX Flow Cytometer (center 1, Yunnan Cancer Hospital, the Third Affiliated Hospital of Kunming Medical University) and BD Biosciences FACS Canto II Flow Cytometer (center 2, the First Affiliated Hospital of Kunming Medical University). Reagents: CD45-FITC, CD3-PC5.5, CD4-PE, Flowcount-PC7 (purchased from BD, USA), hemolysin.

2. Method

(1) Specimen collection: After admission, the patients who met the inclusion criteria were collected 2 ml of fasting venous blood with heparin anticoagulant tube at 6:00-8:00 on the morning. The blood was mixed upside down, and sent for examination within 2 hours.

(2) CD4+ T lymphocyte subsets flow cytometry test: 70ul of anticoagulated whole blood was taken into the sample tube, and the antibody CD45-FITC /CD3-PC5.5 /CD4-PE, 10ul per antibody, were in turn added, which were mixed gently with a mixing, and reacted at room temperature for 15 min in the darkness. Then, 2ml of hemolysin was added to dissolve red blood cells, and reacted again at room temperature for 15 min in the darkness after gently mixing. Finally, 70ul of Flowcount-PC7 for absolute counting and measure by flow cytometry was added to get the percentages and absolute values of CD3, and CD4 in the lymphocyte subsets.

Reference Range of Adult Peripheral Blood CD4+ T cell for Yunnan Province Population in China

Some regional data of lymphocyte phenotypes show variations due to the influence of gender, age, ethnicity, and lifestyle differences.[1-3] These data indicate that different regional populations should have their own defined reference values for peripheral lymphocyte subsets.[1-3] In order to better interpret the results of lymphocyte immunophenotyping in clinical practice, it is necessary to establish a reliable reference value of lymphocyte subsets in healthy people of Yunnan province in China.

We enrolled 631 normal healthy population were recruited among the family members of patients and other volunteers between January 2003 and January 2004 at Yunnan Cancer Center, China.

The enrollment was conducted according to the defined criteria from the SENIEUR protocol guideline.[4] The inclusion criteria was people with age of 18-80 years, and the exclusion criteria was people with testing positive to HIV, systemic infection, connective tissue disease, abnormal tumor marker or cancer.

631 healthy adults included 429 males (67.98%) and 202 females (32.02%). Their mean age was 52. Reference range of T lymphocyte subsets were showed in the Table S3.

Table S1. Reference range of CD4+T Cell in healthy adults of Yunnan province

Parameters	Mean ± SD	95%CI	99%CI
CD3+CD4+ counts (cells/ul)	874 ± 270	345-1402	177-1570
CD3+CD4+/CD3+ (%)	37.6 ± 3.2	31.4-43.8	29.3-45.9

Table S4. Characteristics of colorectal cancer studies between 2005 and July 2019 with focus on the clinical prognostic impact of T cell subsets

Study	Year	*N*	Sides	pStage	Maker	Tumor tissue VS circulating	Validation cohort	Prognostic impact (PI)	PI across each stage/TNM addressed
Zhu et al.[5]	2018	267	Colon and rectum	I-IV	CD8 +	Tumor tissue	Yes	Independent PI	Yes
Pagès et al.[6]	2018	3539	Colon	I-III	CD3+,CD8+	Tumor tissue	Yes	Score of CD3+ and CD8+ independent PI	No
Taylor et al.[7]	2018	95/32	Colon and rectum	I-IV	CD69+ CD4+ IFN-γ-producing CD4+(95) IL-2-producing T cell(32)	Tumor tissue	No	CD69+ CD4+ associated with disease recurrence(95) IFN-γ-producing CD4+ associated with positive patient outcomes(95) IL-2-producing T cell significant PI in univariate with DFS as end point(32)	Yes
Shibutani et al.[8]	2017	90	Colon and rectum	II-III	PD-1+ TILs PD-1/CD8+	Tumor tissue	No	Number of PD-1+ TILs not PI univariate PD-1/CD8+ ratio independent PI	No
Emile et al.[9]	2017	744	Colon	III	CD3+	Tumor tissue	No	Independent PI	stage III only
Yoshida et al.[10]	2016	199	Colon and rectum	II-III	Th1, Th2, Th17, FOXP3+	Tumor tissue	No	Th17/ CD3+ independent PI	No
Kwak et al.[11]	2016	196	Colon and rectum	IV	CD3+, CD4+, CD8+, FOXP3+, CD68+, CD163+	Tumor tissue	No	Score of CD3+ and CD8+ independent PI	Stage IV only
Weixler et al.[12]	2015	657	Colon and rectum	I-III	OX40+/CD8+, OX40+/CD4+,CD4+/CD8+	Tumor tissue	No	OX40+/CD8+ independent PI	No
Ling et al.[13]	2014	278	Colon	I-IV	CD8+, FOXP3(+)	Tumor tissue	No	CD8+ significant PI in univariate FOXP3+ independent PI	Yes
Anitei et al.[14]	2014	111	Rectum	I-IV	CD3+, CD8+	Tumor tissue	No	Score of CD3+ and CD8+ independent PI	Yes
Zeestraten et al.[15]	2013	76	Colon and rectum	I-III	FOXP3+, CD8+/FOXP3+	Tumor tissue	No	CD8+/FOXP3+ independent PI	No
Borda et al.[16]	2012	251	Colon and rectum	I-IV	CD3+	Tumor tissue	No	Score of CD3 and CD8 independent PI	Yes

Dahlin et al.[17]	2011	484	Colon and rectum	I-IV	CD3+	Tumor tissue	No	Independent PI	Yes
Mlecnik et al.[18]	2011	599	Colon and rectum	I-IV	CD8+, CD45RO+	Tumor tissue	Yes	Score of CD45RO+ and CD8+ independent PI	Yes
Tosolini et al.[19]	2011	646	Colon and rectum	I-IV	Th1, Th2, Treg, Th17	Tumor tissue	Yes	Th1, Th17 independent PI	Yes
Suzuki et al.[20]	2010	94	Colon and rectum	I-IV	CD8+, FOXP3+, CD8+ T/FOXP3+	Tumor tissue	No	CD8+ /FOXP3+ independent PI	Yes
Simpson et al.[21]	2010	462	Colon and rectum	I-IV	CD3+	Tumor tissue	No	Independent PI	Yes
Nosho et al.[22]	2010	768	Colon and rectum	I-IV	CD3+, CD8+, CD45RO+, FOXP3+	Tumor tissue	No	CD3+, CD8+, FOXP3+ significant PI in univariate CD45RO+ independent PI	Yes
Sinicrope et al.[23]	2009	160	Colon	II-III	FOXP3+, CD3+, CD3+/FOXP3+	Tumor tissue	No	CD3+/FOXP3+ independent PI CD3+independent PI	No
Qiu et al.[24]	2009	235	Colon and rectum	I-IV	CD3+, CD4+, CD8+, CD4+/CD8+, NK	Circulating	No	CD3+, CD4+/CD8+, NK independent PI	Yes
Laghi et al.[25]	2009	286	Colon and rectum	II-III	CD3+	Tumor tissue	No	Independent PI	No
Pages et al.[26]	2009	602	Colon and rectum	I-II	CD8+, CD45RO+	Tumor tissue	Yes	Score of CD45RO+ and CD8+ independent PI	No
Zlobec et al.[27]	2008	269	Colon	I-III	CD8+	Tumor tissue	No	Independent PI	No
Galon et al.[28]	2006	490	Colon and rectum	I-IV	CD3+, CD8+, GZMB+, CD45RO+	Tumor tissue	Yes	CD3+ independent PI	Yes
Milasiene et al.[29]	2005	40	Colon and rectum	II-IV	CD3+, CD4+, CD8+, CD4+/CD8+, CD20+, CD16+, NK	Circulating	No	Number of CD3+ , CD4+ , CD8 ,CD16+ significant PI in univariate	No

Note: DFS, disease-free survival; IFN, Interferon; IL, interleukin; NK, natural killer; PD1, programmed death-1; PI, prognostic impact; Th, helper T; TILs, tumor-infiltrating lymphocytes;

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