SUPPLEMENTAL

Content:

Table S1: PRISMA checklist.

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	1
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	2
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	4
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	4
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	5
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	5
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	5
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	Table S2, Supplemental
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	6
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	6
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	6
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	8
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	7
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	8

Section/topic	#	Checklist item	Reported on page #
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	8-9
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	None
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	9-10
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	10-13
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	17
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	13-17
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	13-19
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	17
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	None
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	18-19
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	20-21
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	21
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	2

Table S2: Search strategy.

Population	
1	"rectal cancer"
2	"rectum cancer"
3	"rectal carcinoma"
4	"rectal adenocarcinoma"
5	"LARC"
6	"Rectal Neoplasms"
7	#1 OR #2 OR #3 OR #4 OR #5 OR #6
Intervention	
8	"Tumor-infiltrating"
9	"tumour-infiltrating"
10	”TIL”
11	"CD3"
12	"CD8"
13	"FOXP3"
14	"CD4"
15	#8 OR #9 OR #10 OR #11 OR #12 OR #13 OR #14
Comparator	
16	”Density”
17	”Infiltration”
18	”High”
19	”Low”
20	”Responder”
21	”Response”
22	”Good”
23	”Poor”
24	#16 OR #17 OR #18 OR #19 OR #20 OR #21 OR #22 OR #23
Outcome	
25	”Response”
26	”Regression”
27	”pCR”
28	”TRG”
29	”Survival”
30	”OS”
31	”DFS”
32	”RFS”
33	#25 OR #26 OR #27 OR #28 OR #29 OR #30 OR #31 OR #32
Combined search	
34	#7 AND #15 AND #24 AND #33

Table S3: Newcastle-Ottawa Scale - the risk of bias in individual studies.

Study ID	Selection (4)				Comparability (2)	Outcome (3)			Total (9)
	Exposed	Non-exposed	Ascertainment	Outcome		Assessment	Follow-up	Adequacy	
Anitei et al. (5)	1	1	1	1	1	0	1	1	**7**
Yasuda et al. (6)	1	1	1	1	2	1	1	1	**8**
Teng et al. (A) (9)	1	1	1	1	1	1	1	1	**8**
Teng et al. (B) (10)	1	1	1	1	1	1	1	1	**8**
McCoy et al. (11)	1	1	1	1	1	1	1	1	**8**
Shinto et al. (A) (25)	1	1	1	1	2	1	1	1	**9**
Shinto et al. (B) (26)	1	1	1	1	2	0	1	1	**8**
Matsutani et al. (27)	1	1	1	1	1	1	1	1	**8**
Zaghloul et al. (28)	1	1	1	1	1	0	1	1	**7**
Zhang et al. (29)	1	1	1	1	1	1	1	1	**8**
Akiyoshi et al. (30)	1	1	1	1	2	0	1	1	**8**
Chen et al. (31)	1	1	1	1	2	1	1	1	**9**
Moghani et al (32)	1	1	1	1	1	0	1	1	**7**
Xiao et al. (33)	1	1	1	1	2	0	1	1	**8**
Huang, Y et al. (34)	1	1	1	1	2	1	1	1	**9**
Mirjolet et al. (35)	1	1	1	1	1	1	1	1	**8**
Huang, A et al (36)	1	1	1	1	2	1	1	1	**9**
Rudolf et al (37)	1	1	1	1	1	0	1	1	**7**
El-Sissy et al (38)	1	1	1	1	2	1	1	1	**9**

Table S4: GRADE assessment of the quality of evidence for pCR and pTR.

Risk of bias	Indirectness	Inconsistency	Imprecision	Publication bias	Strengths of evidence	Overall quality of evidence
$CD3^+$ TILs and pTR						
Not serious	Not serious	Not serious	Very Serious (-2)	Serious (-1)	None	**-3 = Low**
$CD4^+$ TILs and pTR						
Not serious	Not serious	Not serious	Very Serious (-2)	Serious (-1)	None	**-3 = Low**
$CD8^+$ TILs and pTR						
Not serious	Not serious	Not serious	Not serious	Serious (-1)	None	**-1 = Moderate**
$FoxP3^+$ TILs and pTR						
Not serious	Not serious	Serious (-1)	Very serious (-2)	Serious (-1)	None	**-4 = Very low**
$CD8^+$ TILs and pCR						
Not serious	Not serious	Not serious	Serious (-1)	Serious (-1)	Large effect size (+1)	**-1 = Moderate**

Table S5: GRADE assessment of the quality of evidence for DFS and OS.

Risk of bias	**Indirectness**	**Inconsistency**	**Imprecision**	**Publication bias**	**Strengths of evidence**	**Overall quality of evidence**
$CD8^+$ TILs and DFS						
Not serious	Not serious	Not serious	Not serious	Serious (-1)	None	**-1 = Moderate**
$FoxP3^+$ TILs and DFS						
Not serious	Not serious	Serious (-1)	Very serious (-2)	Serious (-1)	None	**-4 = Very low**
$CD8^+$ TILs and OS						
Not serious	Not serious	Not serious	Serious (-1)	Serious (-1)	Large effect size (+1)	**-1 = Moderate**

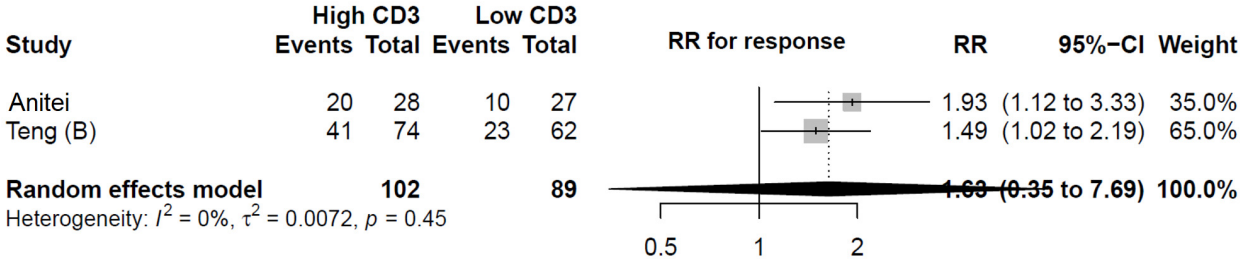

Figure S1: CD3$^+$ TILs density and pTR. A meta-analysis based on random-effects models revealed that a high pretherapeutic CD3$^+$ TILs density was not associated with pTR. RR: Risk Ratio.

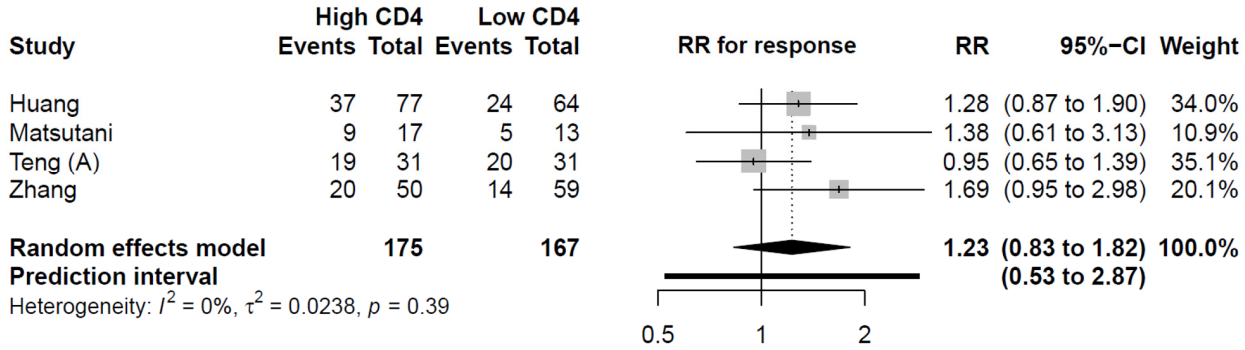

Figure S2: CD4$^+$ TILs density and pTR. A meta-analysis based on random-effects models revealed that a high pretherapeutic CD4$^+$ TILs density was not associated with pTR. RR: Risk Ratio.

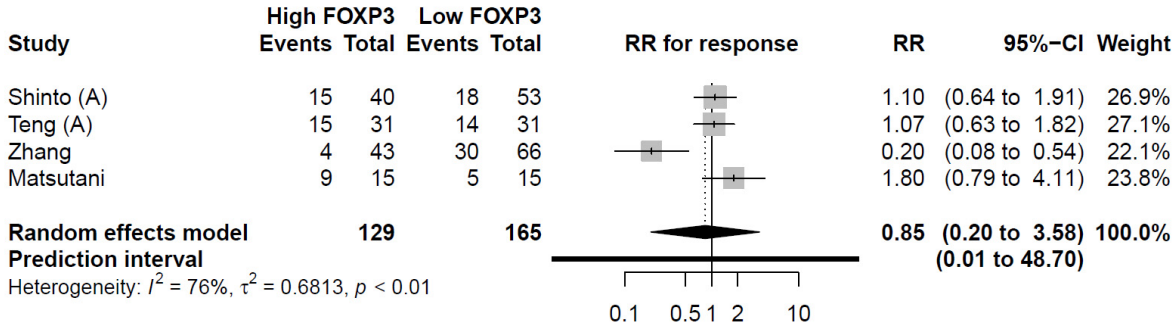

Figure S3: FOXP3$^+$ TILs density and pTR. A meta-analysis based on random-effects models revealed that a high pretherapeutic FOXP3$^+$ TILs density was not associated with pTR. RR: Risk Ratio.

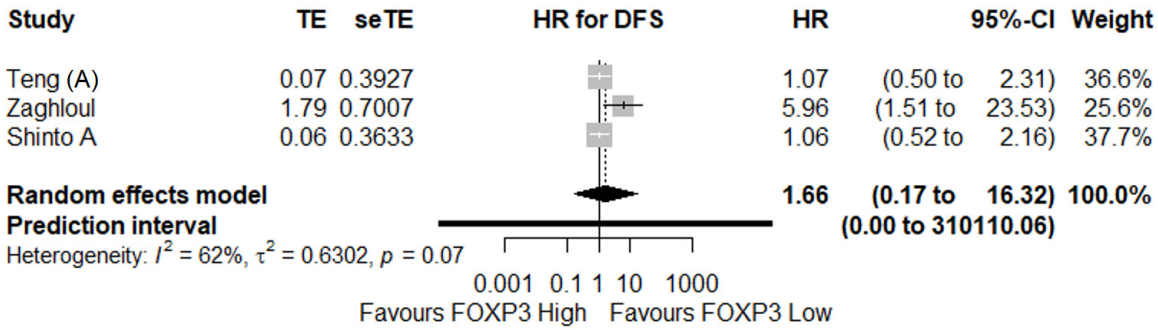

Figure S4: Prognostic value of FOXP3$^+$ TILs. A Meta-analysis of time-to-event data found no association between FOXP3$^+$ TILs density and DFS. HR: Hazard Ratio.