The Prognostic Value of Tumor Infiltrating Lymphocytes After Radical Cystectomy for Bladder Cancer: A Systematic Review and Meta-Analysis

Tatsushi Kawada,[1,2] Takafumi Yanagisawa,[1,3] Pawel Rajwa,[1,4] Reza Sari Motlagh,[1,5] Hadi Mostafaei,[1,6] Fahad Quhal,[1,7] Ekaterina Laukhtina,[1] Maximilian Pallauf,[1,8] Frederik König,[1,9] Benjamin Pradere,[10] Motoo Araki,[2] Yasutomo Nasu,[2] Shahrokh F. Shariat[1,11,12,13,14,15]

Abstract

Background: We aimed to assess the prognostic value of tumor infiltrating lymphocytes (TILs) in patients with bladder cancer (BC) after radical cystectomy (RC). **Materials and Methods:** We searched Pubmed, Web of Science and Scopus in April 2022 to identify studies assessing the prognostic value of TILs, including a subset of lymphocytes (eg, CD3, CD8, FOXP3), after RC. The endpoints were overall survival and recurrent free survival. Subgroup analyses were performed based on the evaluation method for TILs (ie, CD3, CD8, FOXP3, HE staining). **Results:** Overall, 9 studies comprising 1413 patients were included in this meta-analysis. The meta-analysis revealed that elevated expressions of TILs were significantly associated with favorable OS (pooled hazard ratio [HR]: 0.65, 95% confidence interval [CI]: 0.51-0.83) and RFS (pooled HR: 0.48, 95% CI: 0.35-0.64). In subgroup analyses, high CD8+ TILs were also associated with favorable OS (HR: 0.51, 95% CI: 0.33-0.80) and RFS (pooled HR: 0.53, 95% CI: 0.36-0.76). Among 3 studies comprising 146 patients, high intratumoral TILs were significantly associated with favorable OS (pooled HR: 0.34, 95% CI: 0.19-0.60). **Conclusion:** TILs are useful prognostic markers in patients treated with RC for BC. Although the prognostic value of TILs is varied, depending on the subset and infiltration site, CD8+ TILs and intratumoral TILs are associated with oncologic outcomes. Further studies are warranted to explicate the predictive value of TILs on the response to perioperative systemic therapy to help clinical decision-making in patients with BC.

Clinical Genitourinary Cancer, Vol. 22, No. 2, 535–543 © 2024 The Author(s). Published by Elsevier Inc.

Keywords: TIL, MIBC

[1]Department of Urology, Comprehensive Cancer Center, Medical University of Vienna, Vienna, Austria
[2]Department of Urology, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Kita-ku, Okayama, Japan
[3]Department of Urology, The Jikei University School of Medicine, Minato-ku, Tokyo, Japan
[4]Department of Urology, Medical University of Silesia, Zabrze, Poland
[5]Men's Health and Reproductive Health Research Center, Shahid Beheshti University of Medical Sciences, Daneshjoo Blvd, Tehran, Iran
[6]Research Center for Evidence Based Medicine, Tabriz University of Medical Sciences, Tabriz, Iran
[7]Department of Urology, King Fahad Specialist Hospital, Dammam, Saudi Arabia
[8]Department of Urology, Paracelsus Medical University Salzburg, University Hospital Salzburg, Salzburg, Austria
[9]Department of Urology, University Medical Centre Hamburg-Eppendorf, Hamburg, Germany
[10]Department of Urology, La Croix Du Sud Hospital, Quint-Fonsegrives, France
[11]Hourani Center for Applied Scientific Research, Al-Ahliyya Amman University, Amman, Jordan
[12]Department of Urology, University of Texas Southwestern, Dallas, Texas, USA
[13]Department of Urology, Second Faculty of Medicine, Charles University, Prag, Czech Republic
[14]Karl Landsteiner Institute of Urology and Andrology, Vienna, Austria
[15]Research Center for Evidence Medicine, Urology Department Tabriz University of Medical Sciences, Tabriz, Iran

Submitted: Sep 28, 2022; Revised: Jan 7, 2024; Accepted: Jan 9, 2024; Epub: 17 January 2024

Address for correspondence: Shahrokh F. Shariat, MD, Department of Urology, Comprehensive Cancer Center, Medical University of Vienna, Wahringer Gurtel 43 18-20, 1090, Vienna, Austria
E-mail contact: shahrokh.shariat@meduniwien.ac.at

https://doi.org/10.1016/j.clgc.2024.01.008

Introduction

Radical cystectomy (RC) with lymph node dissection preceded by cisplatin-based neoadjuvant chemotherapy (NAC) is the mainstay of treatment for patients with muscle-invasive bladder cancer (MIBC) and very high risk non-muscle invasive bladder cancer (NMIBC). However, despite this strategy, the prognosis of bladder cancer (BC) after RC is still unfavorable and heterogeneous.[1-3] Therefore, there is a need for a well-established biomarker to usher the age of precision medicine in MIBC or at least to help identify the most likely patients to benefit from an intensification of therapy.

BC is a highly immunogenetic tumor as shown by its sensitivity to Bacille Calmette-Guérin (BCG) and immune checkpoint inhibitors (ICI). Evidence related to an important role of the tumor immune microenvironment (TME) in this malignancy is mounting, specifically the association with cancer progression. Tumor infiltrating lymphocytes (TILs) play a crucial role in the TME response to cancer; indeed, several studies reported on TILs to be of prognostic value in various cancers.[4-6] While some studies reported on the prognostic value of TILs in patients with BC, others did not.[7-12] Most studies suffered from a smaller sample size, retrospective single-center design as well as using different evaluation methods for TILs. To overcome these limitations and to assess the prognostic role of TILs in patients with BC after RC, we undertook a systematic review and meta-analysis. Further, we assess how TILs subsets and their location independently affect the prognosis.

Materials and Methods

A protocol for this study was registered a priori on the International Prospective Register of Systematic Reviews (ID: CRD42022310804) (Supplemental Table 1).

Search Strategy

The systematic review and meta-analysis were performed according to the Preferred Reporting Items for Meta-Analyses (PRISMA) of Observational Studies in Epidemiology Statement.[13]

A literature search was conducted using PubMed, Web of Science, and Scopus databases in April 2022 to identify the studies that reported the prognostic value of TILs and survival outcome (OS and RFS) in patients with BC after RC. The search terms were as follows: ("bladder cancer" OR "bladder carcinoma" OR "urothelial cancer" OR "urothelial carcinoma") AND ("tumor infiltrating lymphocytes" OR "lymphocytes tumor infiltrating" OR "TIL" OR "tumor infiltrating immune cells" OR "TIIC").

Initial screening was performed independently by 2 investigators based on the titles and abstracts to identify ineligible reports. Potentially relevant reports were subjected to a full article review and excluded for reasons. Any discrepancies were resolved by a consensus with co-authors.

Inclusion/Exclusion Criteria

Studies were included if they investigated patients with BC who underwent RC (Patients), and had high infiltration of lymphocytes (Intervention) compared to those with low infiltration of lymphocytes (Comparison) regarding oncologic survival outcomes (Outcome) within an observational study design. We included studies assessing TILs by hematoxylin & eosin (HE) staining or immunohistochemistry (IHC) and evaluating overall survival (OS) or Recurrent free survival (RFS) in patients with BC after RC. We only included studies performing multivariable Cox proportional hazard regression analysis to investigate the effect of immune cell infiltration on survival outcomes.

We excluded review articles, letters, editorials, conference abstracts, case reports, nonhuman animal studies, and articles not published in English.

Data Extraction

Two investigators independently extracted the following data: author names, publication year, country of origin, age, number of patients, with or without perioperative chemotherapy, pathological stage, follow-up duration, TILs subset, TILs evaluation method, TILs location, and cut-off value for positive TILs. We included computable data of CD3+ TILs, CD8+ TILs, FOXP3+ TILs, and TILs on HE staining as subsets of TILs in the meta-analysis. Hazard ratio (HR) and 95% confidential intervals (CIs) for OS and RFS based on multivariate Cox hazard analysis were also extracted.

Quality Assessment and Risk of Bias

The risk of bias and applicability were evaluated independently by 2 investigators using the Risk of Bias in nonrandomized Studies of Interventions (ROBINS-I).[14] In ROBINS-I, each bias domain and overall risk of bias was judged as "Low," "Moderate," "Serious," or "Critical" risk of bias. Any discrepancies were resolved by consensus with coauthors.

Statistical Analysis

The primary outcomes were OS and RFS. The pooled HR and 95% CIs were used to analyze the prognostic value of TILs for BC after RC. Cochrane's Q test and I^2 statistics were used to estimate the heterogeneity among the outcomes in this meta-analysis. Significant heterogeneity was indicated by Cochrane's Q test <0.05 and I^2 $>50\%$. If there was significant heterogeneity, a random effect model was used; if not, a fixed effect model was used. Funnel plots were described to assess the publication bias (Supplemental Figure). All analyses were conducted using Review Manager 5.3 (The Cochrane Collaboration, Copenhagen, Denmark); The statistical significance level was set at $P < .05$. We conducted subgroup analyses to account for the heterogeneity of TILs subsets. Each TILs evaluation method was analyzed separately (ie, CD3, CD8, FOXP3, all TILs [HE staining]).

Results

Study Selection and Characteristics

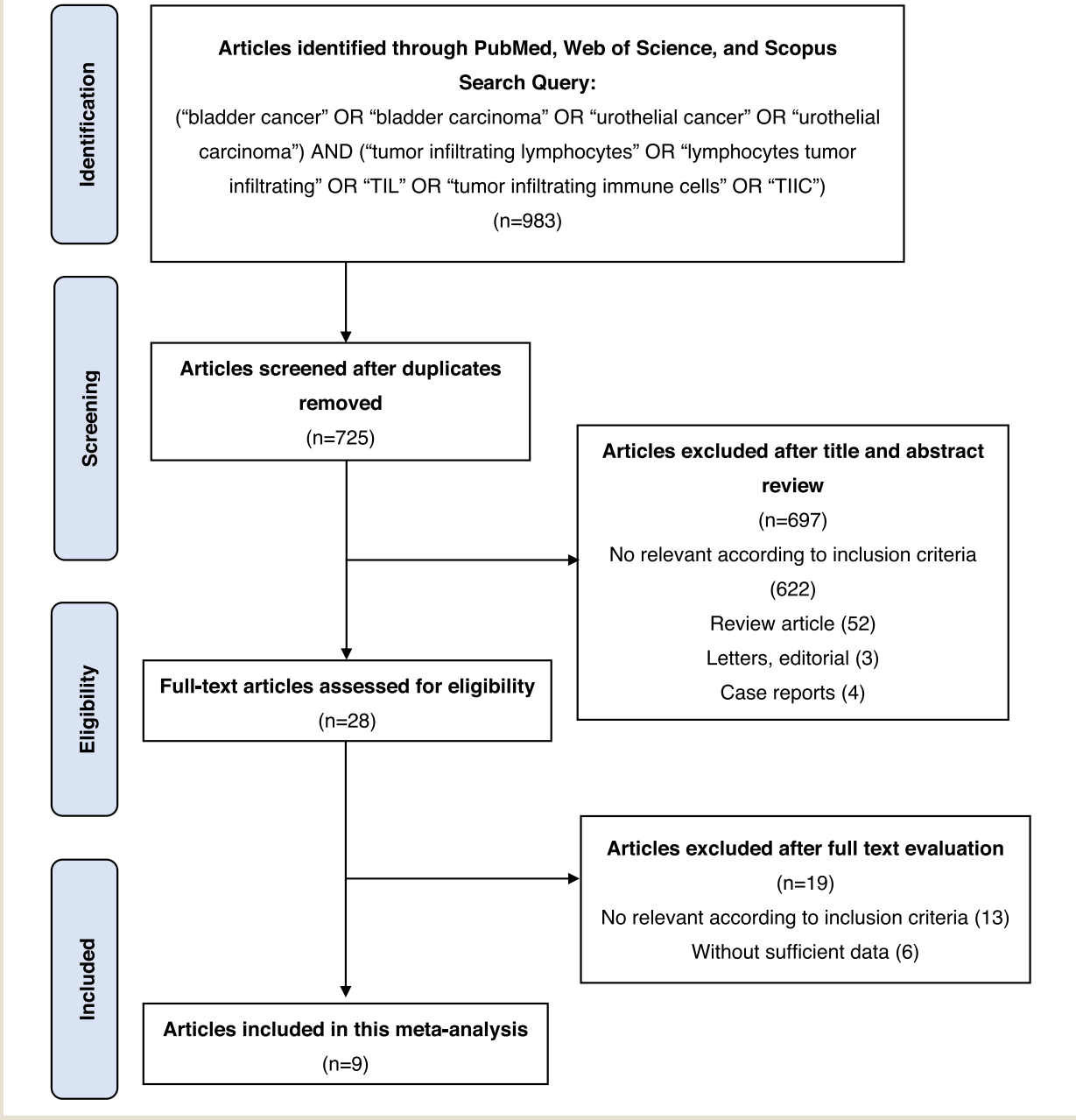
The PRISMA flowchart is presented in Figure 1. In total, 9 studies comprising 1413 patients were included in this meta-analysis.[7-12,15-17] Three studies included only MIBC; the others included patients with both MIBC and high-risk NMIBC. Two studies included patients who underwent cisplatin-based neoadjuvant chemotherapy (NAC), and 6 studies included patients who underwent cisplatin-based adjuvant chemotherapy (ACT). All included studies were retrospective. The characteristics of included studies are shown in Tables 1 and 2.

Table 1 Study Design and Patient's Characteristics

First Author	Publication Year (Year)	Country	Number (n)	Patients Age (Year)	Pathological Stage	Perioperative Chemotherapy	Endpoint	Follow-Up Period (Month)
Sharma	2007	USA	69	median (range) 72(41-90)	≦pT1: 38 (55%) ≧pT2: 31 (45%)	NAC: 0 ACT: 19 (28%)	OS, RFS	median (range) 32 (1-112)
Winerdal	2011	Sweden	37	median (range) 69 (46-81)	≦pT1: 7 (19%) ≧pT2: 30 (81%)	NAC: 0 ACT: 0	OS, RFS	NR
Horn	2016	Germany	149	median (range) 66.2 (35-85)	≦pT1: 18 (12%) ≧pT2: 131 (88%)	NAC: 0 ACT: NR	OS, CSS	median 46
Zhang	2017	China	non-organ-confined:51 organ-confined:75	median non-organ-confined: 63 organ-confined: 60	≦pT1: 65 (52%) ≧pT2: 61 (48%)	NAC: 56 (44%) ACT: 22 (17%)	OS	median non-organ-confined: 30.5 organ-confined: 51.8
Yu	2018	Canada	67	median 67.5	≦pT1: 16 (24%) ≧pT2: 51 (76%)	NAC: 14 (21%) ACT: 13 (19%)	OS, RFS	median 15
Wahlin	2019	Sweden	135	median (range) 71(39-83)	≦pT1: 47 (35%) ≧pT2: 88 (65%)	NAC: 65 (48%) ACT: 12 (9%)	RFS	median (range) 52 (2-95)
Liu	2020	China	cohort 1: 141 cohort 2: 118	median (IQR) cohort 1: 62 (56-71) cohort 2: 62 (55-68)	≧pT2: 259 (100%)	NAC: 0 ACT: 119 (46%)	OS, RFS	NR
Schubert	2020	Germany	320	median (IQR) IIC: 68 (62-73) PIC:68 (59-73) no TILs: 70 (61-76)	≦pT1: 77 (24%) ≧pT2: 243 (76%)	NAC: 0 ACT: 12 (4%)	OS, RFS, CSS	median (range) 37 (10-55)
Sikic	2021	Germany	241	mean ± SD 69±11	≧pT2: 241 (100%)	NAC: 0 ACT: 57 (24%)	OS, RFS, CSS	NR

Abbreviations: TILs = Tumor infiltrating lymphocytes; IIC = Intratumoral immune cell; PIC = Peritumoral immune cell; NAC = Neoadjuvant chemotherapy; ACT = Adjuvant chemotherapy; OS = Overall survival; RFS = Recurrent free survival; CSS = Cancer specific survival; IQR = Interquartile range; SD = Standard deviation; NR = Not reported.

Figure 1 The preferred reporting items for systematic reviews and meta-analyses (PRISMA) flow chart detailing the article selection process.



Applying the ROBINS-I, 3 studies were considered at serious risk, and 6 studies were at moderate risk of bias (Supplemental Table 2). The most affected domain bias was the confounding effect, and this is due to the possible impact of chemotherapy or BCG.

Meta-Analysis for Overall TILs

Eight studies[7,8,10-12,15,17,18] comprised 1268 patients for OS, and 7 studies[7,10-12,15-17] comprised 1128 patients for RFS analysis (Figures 2 and 3). For both analyses, heterogeneities were identified (OS: $I^2 = 66\%$, $P = .001$; RFS: $I^2 = 53\%$, $P = .02$); therefore, random effect models were used. The pooled HRs showed that patients with high levels of TILs had a more favorable OS (HR: 0.65, 95% CI: 0.51-0.83) and RFS (pooled HR: 0.48, 95% CI: 0.35-0.64) compared to those with low levels of TILs (Figures 2 and 3).

Subgroup Analyses for TILs Subsets

CD3+ TILs. In the CD3+ TILs subgroup analyses, we included 3 studies[8,12,15] comprising 253 patients evaluating OS and 2 studies[12,15] comprising 104 patients evaluating RFS. There was no

Table 2 Evaluation Characteristics

First Author	Publication Year (Year)	TILs Subset	TILs Site	Other Markers	Evaluation Method	Cut-Off
Sharma	2007	CD8	intratumoral	NR	IHC	median value
Winerdal	2011	CD3, FOXP3	intratumoral	FOXP3 in tumor cells	IHC	minimum *P*-value analysis
Horn	2016	CD3, CD8, FOXP3	tumor epithelium, peritumoral stroma	FOXP3/CD3 FOXP3/CD8	IHC	minimum *P*-value analysis
Zhang	2017	CD8	intratumoral, stromal	NR	IHC	≧1% stained cells/total cells
Yu	2018	CD3, CD8	Tumor core(CT), invasive margin(IM)	Immune score	IHC	minimum *P*-value analysis
Wahlin	2019	CD8, FOXP3	tumor nest, stromal	CD20, PD-1, PD-L1,	IHC	median value
Liu	2020	CD8	NR	TIGIT+ CD8	IHC	mean value
Schubert	2020	total TILs (HE staining)	IIC, PIC	NR	HE	presence of any immune cell infiltrate
Sikic	2021	total TILs (HE staining)	stromal	NR	HE	≧10% (stromal area occupied by TILs)

Abbreviations: TILs = Tumor infiltrating lymphocytes; CT = Tumor core; IM = Invasive margin; IIC = Intratumorally immune cell; PIC = Peritumorally immune cell; HE = Hematoxylin & eosin; IHC = Immunohistochemistry; NR = Not reported.

Figure 2 Forest plot of the relationship between TILs and OS in patients with BC after RC.

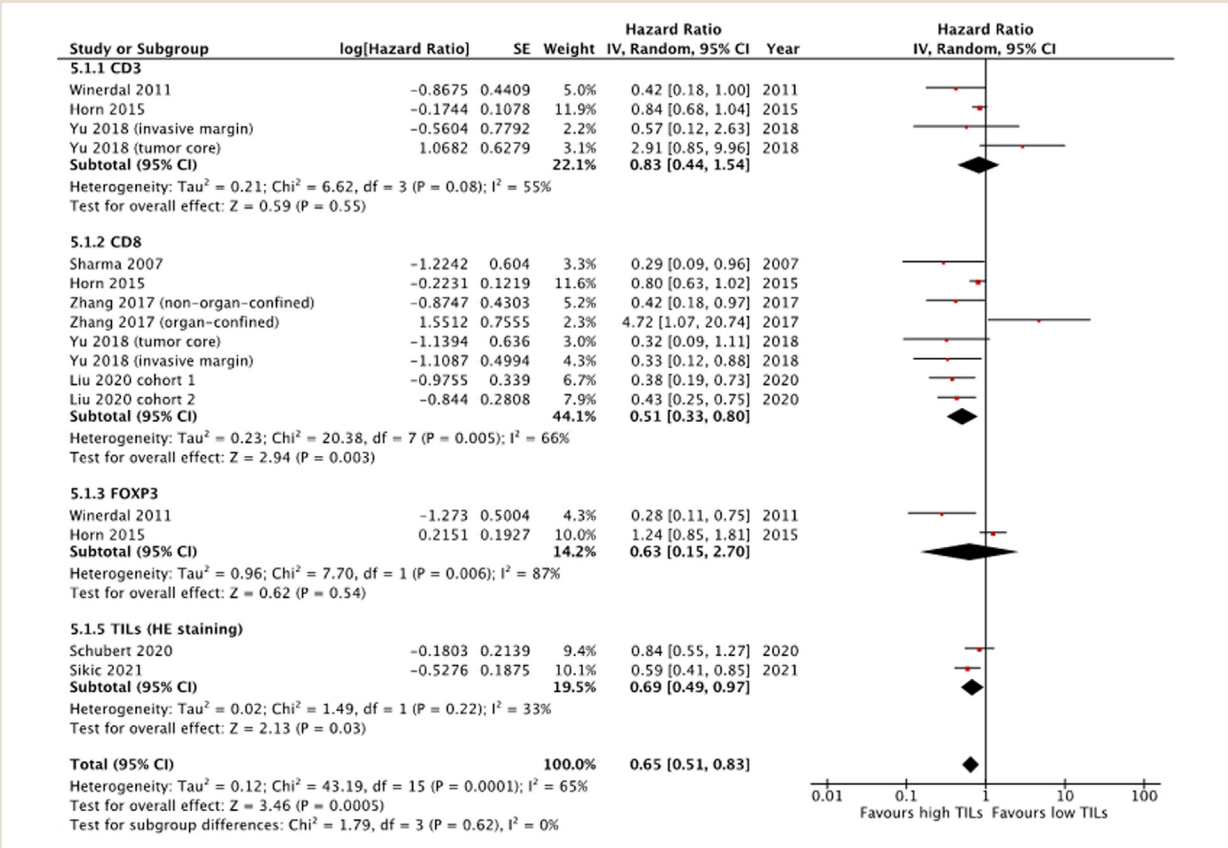
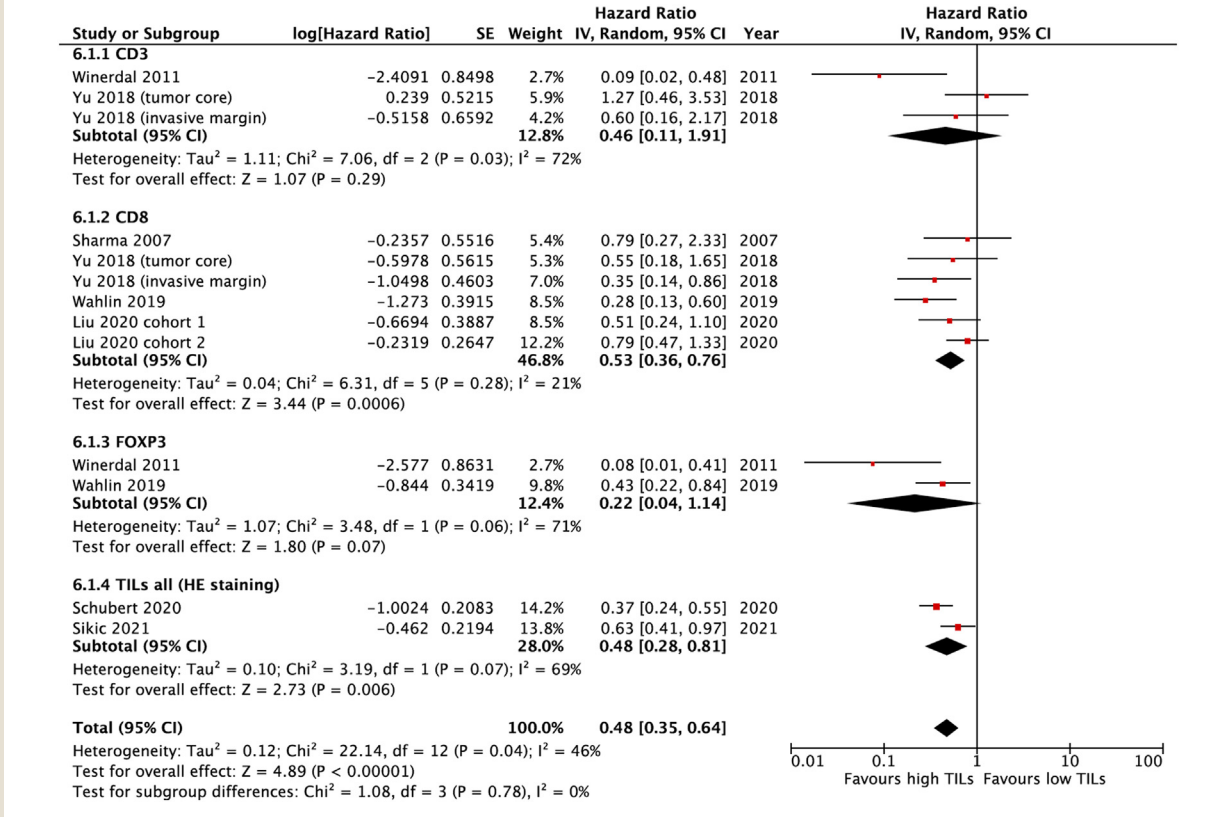


Figure 3 Forest plot of the relationship between TILs and RFS in patients with BC after RC.



significant association of TILs with either OS (HR: 0.83, CI: 0.44-1.54) or RFS (HR: 0.46, CI: 0.11-1.91) (Figures 2 and 3).

CD8+ TILs. In the CD8+ TILs subgroup analyses, we included 5 studies[7,8,10,12,18] comprising 670 patients evaluating OS and 4 studies[7,10,12,16] comprising 540 patients evaluating RFS. The pooled HRs revealed that high levels of CD8+ TILs were associated with both favorable OS (HR: 0.51, CI: 0.33-0.80) and RFS (HR: 0.53, CI: 0.36-0.76) (Figures 2 and 3).

FOXP3+ TILs. In the FOXP3+ TILs subgroup analyses, we included 2 studies[8,15] comprising 186 patients evaluating OS analyses and 2 studies[15,16] comprising 182 patients evaluating RFS. There was no difference in OS (HR: 0.63, CI: 0.15-2.70) and RFS (HR: 0.22, CI: 0.04-1.14) between patients with high and low levels of FOXP3+ TILs (Figures 2 and 3).

TILs on HE Staining. In the TILs on HE staining subgroup analyses, we included 2 studies[11,17] comprising 561 patients evaluating OS and RFS. The pooled HRs showed that high levels of TILs were associated with both favorable OS (pooled HR: 0.69, 95% CI: 0.49-0.97) and RFS (pooled HR: 0.48, 95% CI: 0.28-0.81) (Figures 2 and 3).

Meta-Analysis for Intratumoral TILs

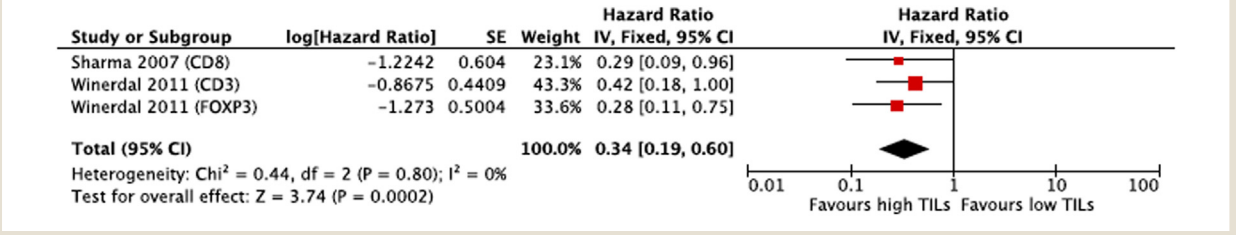
Two studies[7,15] comprising 106 patients were included in the meta-analysis focused on assessing TILs expressed in the intertumoral site as well as its prognostic value for OS. There was no heterogeneity ($I^2 = 0\%$, $P = .80$); therefore, the fixed effect model was used. The pooled HRs showed that high levels of intertumoral TILs were associated with favorable OS (HR: 0.34, 95% CI: 0.19-0.60) (Figure 4).

Discussion

In this meta-analysis, we focused on the prognostic value of TILs in patients with BC after RC. We found that high levels of total and CD8+ TILs were significantly associated with favorable OS and RFS. In contrast, CD3+ TILs and FOXP3+ TILs did not seem to be associated with survival outcomes after RC. Moreover, the pooled data suggest that intrastromal TILs were significantly associated with favorable OS.

Although most of the included studies evaluated the specific subsets of TILs, 2 studies in our meta-analysis evaluated total TILs on HE staining. TILs on HE staining could not be a more specific biomarker than TILs subset but also suggest a prognostic benefit to TILs in patients with BC after RC; TILs are, indeed, practical to measure, and often obtained in the routine setting. A biomarker is needed to be better than what we have today; they must be cheap,

Figure 4 Forest plot of the relationship between intertumoral TILs and OS in patients with BC after RC.



easy, and convenient to utilize.[19] Although the semi-quantitative evaluation method based on HE staining has inherent limitations such as inter-observer variability, previous studies demonstrated that these limitations could be minimalized when the standardized methodology of the international working group on TILs is used.[20-22]

In the subgroup analysis for TILs subsets, the present study showed that high levels of CD8+ TILs were associated with favorable survival outcomes. Since cytotoxic CD8+ T cells are the most powerful effectors in the antitumor immune response, they are likely to have a more precise prognostic value than total TILs. Indeed, most of the included studies that evaluating the prognostic value of CD8+ TILs consistently showed a positive prognostic value for CD8+ TILs, except for 1 study that assessed the prognostic value of CD8+ TILs separately for non-organ confined BC and organ confined BC. These results suggest that the clinical and prognostic value of CD8+ TILs as well as other immunosuppressive cells and receptors, such as regulatory T cells (Tregs) and immune-checkpoint molecules in TME, change according to various factors such as tumor state and stage.

In contrast to CD8+ TILs, we did not find any prognostic value of CD3+ TILs for survival outcomes after RC. The analyzed studies were, indeed, inconsistent similar to the data of other cancers.[4,23,24] One explanation is that CD3+ T cells include not only CD8+ T cells but also CD4+ T cells, which are not associated with tumor behavior. Another explanation is the distribution of TILs. One study out of 3 evaluated the prognostic value of CD3+ TILs with conflicting results from T cell infiltrating site between tumor core (TC) and invasive margin (IM).[12] The study showed the prognostic difference between TC and IM as well as CD3+ TILs and CD8+ TILs. Therefore, considering not only the subset of TILs but also the location of TILs seems to be crucial for determining the prognostic value in patients with BC.

Regulatory T cells (Tregs), the T cell characterized by presence of FOXP3, have a key role in immune tolerance through the function as a suppressor of the immune response in the TME.[25] Associated with this immunosuppressive function, numerous but not all studies reported the relationship between high levels of tumor-infiltrating Tregs and poor prognosis in patients with various cancers.[26,27] Our study included only 2 studies with conflicting results, and we found no significant association between FOXP3+ TILs and survival outcomes. Reasons for these inconsistent results include the other influences on Tregs activation. For instance, the presence of multiple chemokine receptors on Tregs and the different subsets between FOXP3(hi) and FOXP3(lo) T cells have been reported to affect the Treg immunosuppression activity.[28-31] Interestingly, Horn T et. reported that the ratio of FOXP3+ to CD3+ TILs was significantly associated with OS in BC patients who underwent RC .[8] Tregs have a significant role in TME, further analyses are needed to clarify the activation mechanism of their immunosuppressive function and their prognostic value as a biomarker.

Other than TILs subsets, the distribution of TILs are considered as an important factor on the prognostic value in cancers as mentioned above. We also confirmed intratumoral TILs associated with favorable OS in patients with BC after RC. It might be adaptable by the fact that T cells are required physically contact with cancer cells to promote immune response.[32] Although we could not assess the difference on the prognostic value between intratumoral TILs and stromal cells, several studies reported stromal TILs also have a potential as a good prognostic maker in BC patients.[33,34] Further studies are needed to clarify the difference in the effect of immune cells between the locations of TILs (eg, intratumoral or stromal, central tumor or invasive margin) to let the TILs be more clinically useful biomarker.

Our study has several limitations. First, the treatment methods varied among included studies. Although we focused on BC patients who underwent RC, some patients had been treated with chemotherapy and others with BCG that possibly had an influence on the TME. Second, the included studies used different methods and cut-off values to evaluate TILs. Third, we could only perform the subgroup analysis for a limited subset of TILs due to possible positive publication bias. Although some studies reported more specific subsets of TILs or combinations with some other biomarkers, we could not integrate them into our meta-analysis because of the scarcity of the data.[35-37] However, the present systematic review confirmed that total TILs, including those evaluated on HE staining, have a prognostic value for patients treated with RC for BC. Further studies including spatial and time assessment are needed to ensure the clinical value of this biomarker.

Conclusion

The prognostic value of TILs is promising for patients treated with RC for BC. The assessment of TILs on HE slides, especially CD8+ TILs, may help to stratify patients into risk categories for recurrence, thereby helping in the clinical decision-making regarding adjuvant therapy. Further studies are warranted to explicate the

predictive value of TILs on the response to perioperative systemic therapy to help clinical decision-making in patients with bladder cancer.

Ethics Approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Disclosure

All authors state that they have no conflict of interest that might bias this work.

CRediT authorship contribution statement

Tatsushi Kawada: Conceptualization, Data curation, Formal analysis, Investigation, Writing – original draft. **Takafumi Yanagisawa:** Data curation, Formal analysis, Investigation, Writing – review & editing. **Pawel Rajwa:** Writing – review & editing. **Reza Sari Motlagh:** Writing – review & editing. **Hadi Mostafaei:** Writing – review & editing. **Fahad Quhal:** Writing – review & editing. **Ekaterina Laukhtina:** Writing – review & editing. **Maximilian Pallauf:** Writing – review & editing. **Frederik König:** Writing – review & editing. **Benjamin Pradere:** Writing – review & editing. **Motoo Araki:** Writing – review & editing. **Yasutomo Nasu:** Writing – review & editing. **Shahrokh F. Shariat:** Supervision, Writing – review & editing.

Acknowledgments

PR is supported by the EUSP Scholarship of the European Association of Urology (EAU).

References

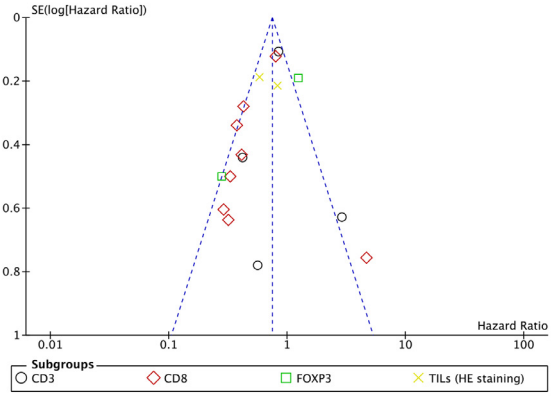
1. Iwata T, Kimura S, Foerster B, et al. Oncologic outcomes after robot-assisted versus open radical cystectomy: a systematic review and meta-analysis. *World J Urol*. 2019;37(8):1557–1570. doi:10.1007/s00345-019-02708-8.
2. Mortezavi A, Crippa A, Edeling S, et al. Morbidity and mortality after robot-assisted radical cystectomy with intracorporeal urinary diversion in octogenarians: results from the European Association of Urology Robotic Urology Section Scientific Working Group. *BJU Int*. 2021;127(5):585–595. doi:10.1111/bju.15274.
3. Yafi FA, Aprikian AG, Chin JL, et al. Contemporary outcomes of 2287 patients with bladder cancer who were treated with radical cystectomy: a Canadian multicentre experience. *BJU Int*. 2011;108(4):539–545. doi:10.1111/j.1464-410X.2010.09912.x.
4. Liu W, Chen G, Zhang C, et al. Prognostic significance of tumor-infiltrating lymphocytes and macrophages in nasopharyngeal carcinoma: a systematic review and meta-analysis. *Eur Arch Otorhinolaryngol*. 2022;279(1):25–35. doi:10.1007/s00405-021-06879-2.
5. Loi S, Michiels S, Adams S, et al. The journey of tumor-infiltrating lymphocytes as a biomarker in breast cancer: clinical utility in an era of checkpoint inhibition. *Ann Oncol*. 2021;32(10):1236–1244. doi:10.1016/j.annonc.2021.07.007.
6. Zheng X, Jin W, Wang S, Ding H. Progression on the roles and mechanisms of tumor-infiltrating T lymphocytes in patients with hepatocellular carcinoma. *Front Immunol*. 2021;12:729705. doi:10.3389/fimmu.2021.729705.
7. Sharma P, Shen Y, Wen S, et al. CD8 tumor-infiltrating lymphocytes are predictive of survival in muscle-invasive urothelial carcinoma. *Proc Natl Acad Sci USA*. 2007;104(10):3967–3972. doi:10.1073/pnas.0611618104.
8. Horn T, Laus J, Seitz AK, et al. The prognostic effect of tumour-infiltrating lymphocytic subpopulations in bladder cancer. *World J Urol*. 2016;34(2):181–187. doi:10.1007/s00345-015-1615-3.
9. Zhang S, Wang J, Zhang X, Zhou F. Tumor-infiltrating CD8+ lymphocytes predict different clinical outcomes in organ- and non-organ-confined urothelial carcinoma of the bladder following radical cystectomy. *PeerJ*. 2017;5:e3921. doi:10.7717/peerj.3921.
10. Liu ZP, Zhou Q, Wang ZW, et al. Intratumoral TIGIT(+)CD8(+)T-cell infiltration determines poor prognosis and immune evasion in patients with muscle-invasive bladder cancer. Article. *J Immunother Cancer*. 2020;8(2):e000978. doi:10.1136/jitc-2020-000978.
11. Sikic D, Weyerer V, Geppert CI, et al. Utility of stromal tumor infiltrating lymphocyte scoring (sTILs) for risk stratification of patients with muscle-invasive urothelial bladder cancer after radical cystectomy. *Urol Oncol*. 2021;40(2):63.e19–63.e26. doi:10.1016/j.urolonc.2021.07.025.
12. Yu A, Mansure JJ, Solanki S, et al. Presence of lymphocytic infiltrate cytotoxic T lymphocyte CD3+, CD8+, and immunoscore as prognostic marker in patients after radical cystectomy. *PLoS One*. 2018;13(10):e0205746. doi:10.1371/journal.pone.0205746.
13. Liberati A, Altman DG, Tetzlaff J, et al. The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate health care interventions: explanation and elaboration. *PLoS Med*. 2009;6(7):e1000100. doi:10.1371/journal.pmed.1000100.
14. Sterne JA, Hernán MA, Reeves BC, et al. ROBINS-I: a tool for assessing risk of bias in non-randomised studies of interventions. *BMJ*. 2016;355:i4919. doi:10.1136/bmj.i4919.
15. Winerdal ME, Marits P, Winerdal M, et al. FOXP3 and survival in urinary bladder cancer. *BJU Int*. 2011;108(10):1672–1678. doi:10.1111/j.1464-410X.2010.10020.x.
16. Wahlin S, Nodin B, Leandersson K, Boman K, Jirström K. Clinical impact of T cells, B cells and the PD-1/PD-L1 pathway in muscle invasive bladder cancer: a comparative study of transurethral resection and cystectomy specimens. *Oncoimmunology*. 2019;8(11):e1644108. doi:10.1080/2162402x.2019.1644108.
17. Schubert T, Renninger M, Schmid MA, et al. Prognostic impact of tumor-associated immune cell infiltrates at radical cystectomy for bladder cancer. *Urol Oncol-Semin Orig Investig*. 2020;38(1):4.e7–4.e15. doi:10.1016/j.urolonc.2019.08.013.
18. Zhang QL, Hao CL, Cheng GZ, et al. High CD4(+) T cell density is associated with poor prognosis in patients with non-muscle-invasive bladder cancer. *Int J Clin Exp Pathol*. 2015;8(9):11510–11516.
19. Bensalah K, Montorsi F, Shariat SF. Challenges of cancer biomarker profiling. *Eur Urol*. 2007;52(6):1601–1609. doi:10.1016/j.eururo.2007.09.036.
20. O'Loughlin M, Andreu X, Bianchi S, et al. Reproducibility and predictive value of scoring stromal tumour infiltrating lymphocytes in triple-negative breast cancer: a multi-institutional study. *Breast Cancer Res Treat*. 2018;171(1):1–9. doi:10.1007/s10549-018-4825-8.
21. Hendry S, Salgado R, Gevaert T, et al. Assessing tumor-infiltrating lymphocytes in solid tumors: a practical review for pathologists and proposal for a standardized method from the international immunooncology biomarkers working group: part 1: assessing the host immune response, TILs in invasive breast carcinoma and ductal carcinoma in situ, metastatic tumor deposits and areas for further research. *Adv Anat Pathol*. 2017;24(5):235–251. doi:10.1097/pap.0000000000000162.
22. Hendry S, Salgado R, Gevaert T, et al. Assessing tumor-infiltrating lymphocytes in solid tumors: a practical review for pathologists and proposal for a standardized method from the international immuno-oncology biomarkers working group: part 2: TILs in melanoma, gastrointestinal tract carcinomas, non-small cell lung carcinoma and mesothelioma, endometrial and ovarian carcinomas, squamous cell carcinoma of the head and neck, genitourinary carcinomas, and primary brain tumors. *Adv Anat Pathol*. 2017;24(6):311–335. doi:10.1097/pap.0000000000000161.
23. Berele BA, Cai Y, Yang G. Prognostic value of tumor infiltrating lymphocytes in nasopharyngeal carcinoma patients: meta-analysis. *Technol Cancer Res Treat*. 2021;20:15330338211034265. doi:10.1177/15330338211034265.
24. Schoenberg MB, Li X, Li X, et al. The predictive value of tumor infiltrating leukocytes in hepatocellular carcinoma: a systematic review and meta-analysis. *Eur J Surg Oncol*. 2021;47(10):2561–2570. doi:10.1016/j.ejso.2021.04.042.
25. Kim JH, Kim BS, Lee SK. Regulatory T cells in tumor microenvironment and approach for anticancer immunotherapy. *Immune Netw*. 2020;20(1):e4. doi:10.4110/in.2020.20.e4.
26. Shang B, Liu Y, Jiang SJ, Liu Y. Prognostic value of tumor-infiltrating FoxP3+ regulatory T cells in cancers: a systematic review and meta-analysis. *Sci Rep*. 2015;5:15179. doi:10.1038/srep15179.
27. Salama P, Phillips M, Grieu F, et al. Tumor-infiltrating FOXP3+ T regulatory cells show strong prognostic significance in colorectal cancer. *J Clin Oncol*. 2009;27(2):186–192. doi:10.1200/jco.2008.18.7229.
28. Akimova T, Zhang T, Negorev D, et al. Human lung tumor FOXP3+ Tregs upregulate four "Treg-locking" transcription factors. *JCI Insight*. 2017;2(16):e94075. doi:10.1172/jci.insight.94075.
29. Lee I, Wang L, Wells AD, Dorf ME, Ozkaynak E, Hancock WW. Recruitment of Foxp3+ T regulatory cells mediating allograft tolerance depends on the CCR4 chemokine receptor. *J Exp Med*. 2005;201(7):1037–1044. doi:10.1084/jem.20041709.
30. Lv M, Xu Y, Tang R, et al. miR141-CXCL1-CXCR2 signaling-induced Treg recruitment regulates metastases and survival of non-small cell lung cancer. *Mol Cancer Ther*. 2014;13(12):3152–3162. doi:10.1158/1535-7163.Mct-14-0448.
31. Saito T, Nishikawa H, Wada H, et al. Two FOXP3(+)CD4(+) T cell subpopulations distinctly control the prognosis of colorectal cancers. *Nat Med*. 2016;22(6):679–684. doi:10.1038/nm.4086.
32. Joyce JA, Fearon DT. T cell exclusion, immune privilege, and the tumor microenvironment. *Science*. 2015;348(6230):74–80. doi:10.1126/science.aaa6204.

33. Pfannstiel C, Strissel PL, Chiappinelli KB, et al. The tumor immune microenvironment drives a prognostic relevance that correlates with bladder cancer subtypes. *Cancer Immunol Res*. 2019;7(6):923–938. doi:10.1158/2326-6066.Cir-18-0758.
34. Rouanne M, Betari R, Radulescu C, et al. Stromal lymphocyte infiltration is associated with tumour invasion depth but is not prognostic in high-grade T1 bladder cancer. *Eur J Cancer*. 2019;108:111–119. doi:10.1016/j.ejca.2018.12.010.
35. Huang Q, Zhou Q, Zhang H, et al. Identification and validation of an excellent prognosis subtype of muscle-invasive bladder cancer patients with intratumoral CXCR5(+) CD8(+) T cell abundance. *Oncoimmunology*. 2020;9(1):1810489. doi:10.1080/2162402x.2020.1810489.
36. Kim H, Kim O, Lee MA, et al. Prognostic impact of APOBEC3B expression in metastatic urothelial carcinoma and its association with tumor-infiltrating cytotoxic T cells. *Curr Oncol*. 2021;28(3):1652–1662. doi:10.3390/curroncol28030154.
37. Zhong Q, Shou J, Ying J, et al. High PD-L1 expression on immune cells, but not on tumor cells, is a favorable prognostic factor in urothelial carcinoma. *Future Oncol*. 2021;17(22):2893–2905. doi:10.2217/fon-2021-0092.

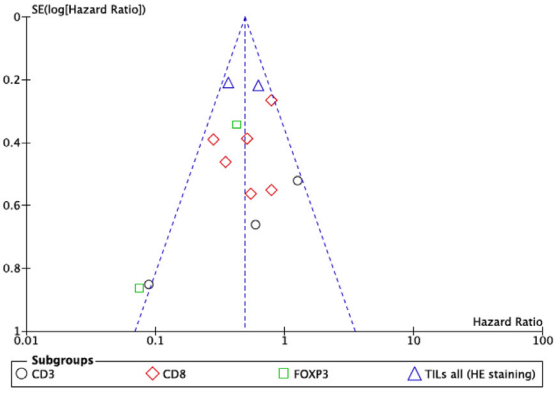
Supplementary materials

Supplemental Figure Funnel plot of included studies.

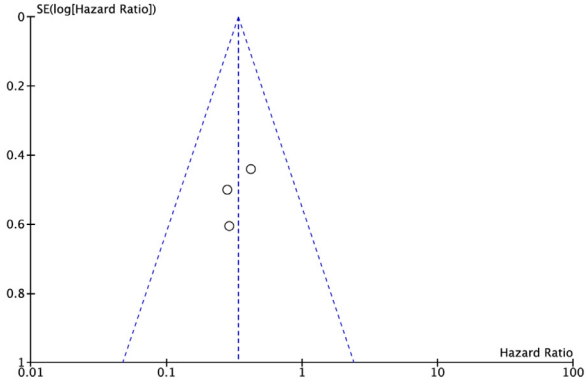
(A) Funnel plot of studies on TILs and OS



(B) Funnel plot of studies on TILs and RFS



(C) Funnel plot of studies on intratumoral TILs and OS



Supplemental Table 1 **PRISMA Checklist 2009**

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.	3,4
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	3,4, and 6
Methods			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (eg, Web address), and, if available, provide registration information including registration number.	5
Eligibility criteria	6	Specify study characteristics (eg, PICOS, length of follow-up) and report characteristics (eg, years considered, language, publication status) used as criteria for eligibility, giving rationale.	5,6
Information sources	7	Describe all information sources (eg, databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	5,6
Search	8	Present full electronic search strategy for at least 1 database, including any limits used, such that it could be repeated.	5,6
Study selection	9	State the process for selecting studies (ie, screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	5,6 and Figure. 1
Data collection process	10	Describe method of data extraction from reports (eg, 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 (eg, 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.	7

(*continued on next page*)

Supplemental Table 1 (*continued*)

Section/Topic	#	Checklist Item	Reported on Page #
Summary measures	13	State the principal summary measures (eg, 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 (eg, I2) for each meta-analysis.	7
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (eg, publication bias, selective reporting within studies).	7
Additional analyses	16	Describe methods of additional analyses (eg, 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.	8, Figure 1
Study characteristics	18	For each study, present characteristics for which data were extracted (eg, study size, PICOS, follow-up period) and provide the citations.	8, Tables 1 and 2
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).	8, Table S1
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.	9 to 10
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	9 to 10
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	Table S1
Additional analysis	23	Give results of additional analyses, if done (eg, 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 (eg, healthcare providers, users, and policy makers).	11 to 14
Limitations	25	Discuss limitations at study and outcome level (eg, risk of bias), and at review-level (eg, incomplete retrieval of identified research, reporting bias).	13 to 14
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	14
Funding			
Funding	27	Describe sources of funding for the systematic review and other support (eg, supply of data); role of funders for the systematic review.	14

Supplemental Table 2 Risk of Bias Assessment for NRCTs (ROBINS-I)

Study	Year	Confounding	Paticipants' Selection	Classification of Interventions	Deviations From Intended Intervention	Missing Data	Measurement of Outcomes	Selection of the Reported Result	Overall
Sharma	2007	Moderate	Low	Moderate	Low	Low	Low	Low	Moderate
Winerdal	2011	Moderate	Low	Moderate	Low	Low	Low	Low	Moderate
Horn	2016	Moderate	Low	Low	Low	Low	Low	Low	Moderate
Zhang	2017	Serious	Low	Low	Low	Low	Low	Low	Serious
Yu	2018	Serious	Low	Low	Low	Low	Low	Low	Serious
Wahlin	2019	Serious	Low	Moderate	Low	Low	Low	Low	Serious
Liu	2020	Moderate	Moderate	Low	Low	Low	Low	Moderate	Moderate
Schubert	2020	Moderate	Low	Moderate	Low	Low	Low	Low	Moderate
Sikic	2021	Moderate	Moderate	Low	Low	Low	Low	Low	Moderate

Abbreviations: NRCTs = nonrandomized comparative studies; ROBINS-I = risk of bias in nonrandomized studies -of interventions.