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## Research Paper

# The association between CD8+ tumor-infiltrating lymphocytes and the clinical outcome of cancer immunotherapy: A systematic review and meta-analysis

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## ABSTRACT

**Background:** The responses of cancer patients to immune checkpoint inhibitors (ICIs) vary in success. CD8+ tumor infiltrating lymphocytes (TILs) play a key role in killing tumor cells. This study aims to evaluate the prognostic role of CD8+ TILs in cancer patients treated with ICIs.

**Methods:** We systematically searched all publications from PubMed, EMBASE, and Cochrane Library until 12 Jul 2021 without any restriction of language or article types. Studies assessing high versus low CD8+ TILs in predicting efficacy and survival of various cancer patients were included. The outcomes included overall survival (OS), progression-free survival (PFS), and objective response rate (ORR). The study protocol is prospectively registered on PROSPERO (registration number CRD42021233654).

**Findings:** Findings: A total of 33 studies consisting of 2559 cancer patients were included. The result showed that high CD8+ TILs were significantly associated with better OS (HR, 0.52; 95% confidence interval: 0.41–0.67;  $p < 0.001$ ), PFS (HR, 0.52; 95% confidence interval: 0.40–0.67;  $p < 0.001$ ) and ORR (OR, 4.08; 95% confidence interval: 2.73–6.10;  $p < 0.001$ ) in patients treated with ICIs. Subgroup analyses suggested that patients with high CD8+ TILs had a better clinical benefit, regardless of different treatments (ICI mono therapy, or combination therapy), cancer types (NSCLC, melanoma and others), and CD8+ T cells locations (intra-tumor, stroma, and invasive margin). The higher baseline circulating CD8+ T cells from peripheral blood did not contribute to the improved OS (HR, 0.93; 95% confidence interval: 0.67–1.29;  $p = 0.67$ ) and PFS (HR, 0.89; 95% confidence interval: 0.60–1.32;  $p = 0.56$ ) compared with the low baseline.

**Interpretation:** Interpretation: Our results suggested that high intra-tumoral, stromal, or invasive marginal, but not circulating CD8+ T cells, can predict treatment outcomes in patients with ICIs therapy across different cancers, in either single-agent ICIs or combination with other therapies.

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## 1. Introduction

Immune checkpoint inhibitors (ICIs) have revolutionized cancer treatments. PD-1/PD-L1 inhibitors can block PD-1/PD-L1 interaction, which is known to drive T cells dysfunction, and release the brake on T cell anti-tumor immune responses [1,2]. However, the responses of

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## Research in context

### Evidence before this study

Effective immunotherapy requires thorough knowledge of the tumor microenvironment. It had been shown that the presence of high CD8<sup>+</sup> TILs contributed to longer survival in cancer patients received ICIs treatment. However, some articles had conflicting and inconclusive evidence. In addition, the metabolic regulation, the functional states, the subtype, and the spatial distribution of CD8<sup>+</sup> T cells play different roles in predicting prognosis in patients received ICIs. We aimed to clarify the prognostic value of CD8<sup>+</sup> TILs on OS, PFS, and ORR in various cancer patients treated with ICIs.

### Added value of this study

A total of 33 studies consisting of 2559 cancer patients were included. To the best of our knowledge, this is the first meta-analysis which showed that high CD8<sup>+</sup> T cells in tissue, but not in peripheral blood could predict better prognosis in patient with ICIs therapy, across different cancers.

### Implication of all the available evidence

This study suggested that the density of CD8<sup>+</sup> TILs should be taken into account before cancer patients received ICIs treatment. Pre-assessment of the density and location of CD8<sup>+</sup> T cells may promote individualized immunotherapy outcomes. Patients with high CD8<sup>+</sup> TILs had better clinical outcomes.

## 2. Methods

### 2.1. Search strategy and selection criteria

This meta-analysis was conducted in accordance with PRISMA (preferred reporting items for systematic reviews and meta-analyses) guidelines [14]. The protocol was registered in the Prospective Register of Systematic Reviews (PROSPERO CRD42021233654). The study was exempted from review by the institutional review board for the innocuousness of this study.

We systematically searched all publications from PubMed, EMBASE, and Cochrane Library until 12 Jul 2021 without any restriction of language or article types. Following keywords and Medical Subject Headings (MeSH) terms were contained: immune checkpoint inhibitors, cytotoxic T-lymphocyte-associated protein 4, programmed death-ligand 1, programmed death receptor 1, CD8<sup>+</sup> tumor-infiltrating lymphocytes, and carcinoma (eTable 1 in the appendix). Furthermore, we manually searched recommended references from systematic reviews, meta-analyses, and conference proceedings.

Studies assessing high versus low CD8<sup>+</sup> TILs in predicting efficacy and survival of various cancer patients treated with ICIs were considered. The inclusion criteria were as follows: 1) Patients: advanced or metastatic cancer patients diagnosed by cytology or pathology. 2) Study type: observational (cohort, case-control, and cross-sectional with binary outcomes) or interventional studies (randomized controlled trials). 3) Intervention: ICIs (anti-PD-1, anti-PD-L1, and anti-CTLA-4 inhibitors) with or without other therapies. 4) Biomarker: CD8<sup>+</sup> T cells derived from tumor tissues or peripheral blood. 5) Outcome: available data that measured OS, PFS, or ORR. The ORR was defined as the sum of complete response (CR) and partial response (PR), assessed by RECIST or irRC. The exclusion criteria were as follows: studies with insufficient data, reviews, notes, letters, editorials, comments, case reports, expert opinions and animal studies.

The following data were extracted: baseline characteristic of each study (author, year, study type, country), patients characteristics (median age, gender, number, cancer type and treatments), information of CD8<sup>+</sup> T cells (detection method, sample type, location, and cutoff value), outcomes (ORR, PFS, OS) and their statistics values (HR, OR, 95% CI).

All included articles were independently selected by two authors (FL and LQZ). The process of data extraction and quality assessment were performed by SX and JFL independently. Any discrepancies were resolved by discussion by a panel of adjudicators (FL, XYZ, ZHX, LQZ, CCL, BC, SX, JFL, RZ, ZXC, and ZWY).

### 2.2. Data analysis

The software Stata version 16 MP (Stata Corporation, College Station, TX, USA) was used to perform the meta-analysis. When uni-variate and multivariate analysis were performed for HRs and its 95% CIs, the latter analysis was chosen. If there were Kaplan–Meier curves without specific HR value in the study, HRs were calculated following the method previously described [15,16].

The Cochrane's Q test (chi-squared test; Chi2) and I<sup>2</sup> value were used to assess the magnitude of heterogeneity among the included studies. The pooled estimates of HRs and 95% CIs were calculated using the random-effects inverse-variance-weighted model, while OR and 95%CI were calculated using the random-effects DerSimonian-Laird model. The subgroup analyses of OS, PFS, and ORR were performed in terms of treatment types, cancer types, and CD8<sup>+</sup> T cells location. The cumulative meta-analysis was conducted based on the year of publication. Sensitivity analysis was also performed to explore the possible source of heterogeneity. Funnel plot analysis and Egger's test were performed to assess publication bias. It would be defined as statistically significant heterogeneity when

cancer patients to ICIs vary in success. Unmet needs exist in predicting such responses with accurate biomarkers to maximize the efficacy and minimize the toxicity of ICIs.

The tools of evaluation on ICIs response have evolved from imaging to molecular or genetic alteration. Biomarkers deriving from tumor immune microenvironment and tumor cell-intrinsic features, such as PD-L1 expression status, tumor mutational burden (TMB), tumor-infiltrating lymphocytes (TILs) and mismatch-repair (MMR) deficiency, were reported to be correlated with the effect of ICIs treatment [3]. ICIs could overcome the dysfunction and exhaustion of T cells resulting from transcriptional and translational regulation of the various cell populations in the tumor microenvironment (TME) [4]. CD8<sup>+</sup> TILs are critical determinant of response to ICIs treatment since their direct role in tumor cell destruction [5,6]. Dann et.al demonstrated that the presence of high CD8<sup>+</sup> TILs were a potential biomarker to predict a better PFS in NSCLC patients receiving Nivolumab [7]. Leisha et al. showed that a higher ORR and a longer PFS and OS were observed in triple-negative breast cancer patients with higher CD8<sup>+</sup> TILs before atezolizumab therapy [8]. However, Sylvia et al. proposed that there was no statistically significant association of CD8<sup>+</sup> TILs density with clinical outcome [9]. In addition, the metabolic regulation, the functional states [4], the subtype, and spatial distribution of CD8<sup>+</sup> T cells play different roles in tumor immunity [10–13]. The effect of CD8<sup>+</sup> T cells to immunotherapy is still in debate.

We herein performed a comprehensive pooled analysis to clarify the prognostic value of CD8<sup>+</sup> TILs on OS, PFS, and ORR in various cancer patients treated with ICIs. Subgroup analyses by different treatments (ICIs mono therapy and combination therapy), cancer types (NSCLC, melanoma and others), and CD8<sup>+</sup> T cells locations (intra-tumor, stroma, and invasive margin) were conducted. We also explored the role of circulating CD8<sup>+</sup> T cells from peripheral blood.

chi-squared  $p$ -value  $< 0.1$  or an  $I^2$  statistic  $> 50\%$ . For all pooled analyses, a  $p$ -value less than 0.05 suggested a statistical significance.

### 2.3. Role of the funding source

The funder of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

## 3. Result

### 3.1. Characteristics and quality of the included studies

A total of 5123 articles were obtained through the initial search strategy. After screening abstract and reviewing full texts, 33 articles [7–9,17–46] published from 2014 to 2021 were considered as eligible in final analyses (Fig. 1A).

The baseline characteristics of all included articles were listed in Table 1. A total of 2559 patients diagnosed with non-small cell lung cancer, melanoma, and other solid tumors, who received ICIs mono therapy or ICIs combination therapy, were enrolled. CD8+ T cells were derived from tumor tissue (28/33 studies) or peripheral blood (5/33 studies). CD8+ T cells in tissue come from primary tumor rather than the metastasis. This distinction is crucial, as the strength of the immune system decreases in the metastatic setting. The baseline CD8+ TILs in tissue before ICIs treatments were examined in 3 compartments: intra-tumor, stroma and invasive margin. The cutoff value for defining high and low CD8+ T cells was according to the each included study independently.

The Newcastle-Ottawa Scale (NOS) was used to assess the quality of included studies. Three quality parameters: selection, comparability, and outcomes were mainly consisted according to NOS. There are four, one and three criteria items in concerns of selection, comparability and outcome, independently. A score with more than six was considered as high quality. 87.9% of the included studies were considered high quality (Fig. 1B). Four studies were considered as low quality. Selection and outcome bias were the main reasons for lowering the overall quality.

### 3.2 Correlation between CD8+ tumor infiltration lymphocytes and prognosis

As is shown in Fig. 2A, the pooled results revealed that patients with high CD8+ TILs exhibited longer OS, compared with those with low CD8+ TILs (HR, 0.52; 95% CI: 0.41–0.67;  $p < 0.001$ ). In terms of PFS, high CD8+ TILs led to 48% reduction in the risk of disease progression compared with low CD8+ TILs (HR, 0.52; 95% CI: 0.40–0.67;  $p < 0.001$ ) (Fig. 2B). The cumulative analysis of the pooled OS and PFS showed a good reliance (eFigure 1 in the appendix). Significant heterogeneity was observed in OS ( $I^2 = 76.34\%$ ,  $\chi^2 = 71.85$ ,  $p < 0.001$ ) and PFS ( $I^2 = 70.65\%$ ,  $\chi^2 = 51.11$ ,  $p < 0.001$ ). In addition, high CD8+ TILs were associated with significant higher objective response rate rather than low CD8+ TILs (OR = 4.08; 95% CI: 2.73–6.10;  $p < 0.001$ ), and no significant heterogeneity was observed ( $I^2 = 24.45\%$ ,  $\chi^2 = 25.15$ ,  $p = 0.16$ ) (Fig. 2C).

### 3.3. Subgroup analyses by treatments

We examined the impact of ICIs mono therapy and ICIs combination therapy. For patient with high CD8+TILs, the pooled HR for OS was 0.51 (95% CI: 0.39–0.66;  $p < 0.001$ ; heterogeneity,  $p < 0.001$ ) for patients with ICIs mono therapy, and non-statistically significant HR of 0.45 (95% CI: 0.12–1.66;  $p = 0.233$ ) in those with ICIs-combination therapy (Fig. 3A; e Figure 2A in the appendix). High CD8+ TILs

were associated with significant better PFS in ICIs combination subgroup, with the HR of 0.27 (95% CI: 0.09–0.81;  $p = 0.019$ ; heterogeneity,  $p = 0.22$ ) for ICIs combination subgroup while 0.52 (95% CI: 0.40–0.68;  $p < 0.001$ ; heterogeneity,  $p < 0.001$ ) for ICIs mono therapy subgroup (Fig. 3B; e Figure 2B in the appendix). The combined OR of the ICIs mono therapy subgroup was 4.69 (95%CI: 3.01–7.28;  $p < 0.001$ ; Heterogeneity,  $p = 0.19$ ), and 2.19 (95%CI: 0.89–5.40;  $p = 0.88$ ; Heterogeneity,  $p = 0.31$ ) in the ICIs combination therapy subgroup (Fig. 3C; eFigure 2C in the appendix).

### 3.4. Subgroup analyses by cancer types

When classified by cancer types, high CD8+ TILs of NSCLC (HR, 0.55; 95%CI: 0.39–0.77;  $p = 0.001$ ; heterogeneity,  $p < 0.001$ ) and other solid tumor (HR, 0.54; 95%CI: 0.43–0.67;  $p < 0.001$ ; heterogeneity,  $p = 0.46$ ) were associated with improved OS, while no statistically significant improvement was reported in melanoma (HR, 0.45; 95%CI: 0.18–1.13;  $p = 0.088$ ; heterogeneity,  $p = 0.17$ ) (Fig. 3A; eFigure 3A in the appendix). The HRs for PFS were 0.57 (95% CI: 0.39–0.83;  $p = 0.003$ ; heterogeneity,  $p < 0.001$ ), 0.38 (95% CI: 0.25–0.58;  $p < 0.001$ ; heterogeneity,  $p = 0.49$ ), 0.57 (95% CI: 0.43–0.75;  $p < 0.001$ ; heterogeneity,  $p = 0.34$ ) in NSCLC, melanoma and other cancers, suggesting that longer PFS in patients with high CD8+ TILs (Fig. 3B; eFigure 3B in the appendix), regardless of cancer types. For NSCLC (OR, 4.04; 95% CI: 2.28–7.15;  $p < 0.001$ ; Heterogeneity,  $p = 0.35$ ), melanoma (OR, 6.02; 95% CI: 2.72–13.31;  $p < 0.001$ ; Heterogeneity,  $p = 0.22$ ) and other cancers (OR, 2.57; 95% CI: 1.38–4.77;  $p < 0.001$ ; Heterogeneity,  $p = 0.34$ ), patients with high CD8+ TILs had higher ORR (Fig. 3C; eFigure 3C in the appendix).

### 3.5. Subgroup analyses by CD8+ t cells location

The presence of CD8+ T cells in different locations has also been proposed as a biomarker for ICI efficacy. The pooled analysis revealed that patient with high CD8+ T cells in total intra-tumor and stroma (HR, 0.53; 95% CI: 0.41–0.68;  $p < 0.001$ ; heterogeneity,  $p = 0.73$ ), intra-tumor (HR, 0.59; 95% CI: 0.40–0.86;  $p = 0.007$ ; heterogeneity,  $p < 0.001$ ) or stroma (HR, 0.41; 95% CI: 0.29–0.58;  $p < 0.001$ ; heterogeneity,  $p < 0.001$ ) had better OS (Fig. 3A; eFigure 4A in the appendix). Similar benefit for PFS was also observed. High CD8+ T cells infiltration with an enhanced PFS exist in intra-tumor and stroma (HR, 0.51; 95% CI: 0.33–0.79;  $p = 0.003$ ; heterogeneity,  $p = 0.05$ ), intra-tumor (HR, 0.61; 95% CI: 0.41–0.92;  $p = 0.017$ ; heterogeneity,  $p = 0.01$ ) and stroma (HR, 0.49; 95% CI: 0.33–0.71;  $p < 0.001$ ; heterogeneity,  $p = 0.61$ ), respectively (Fig. 3B; eFigure 4B in the appendix). In addition, our analyses suggested that the presence of stromal CD8+ TILs was a stronger biomarker for PFS and OS than intra-tumoral CD8+ TILs. Data on predictive value of CD8+ T cells in the invasive margin were limited and only the pooled ORR was performed due to the lack of data on OS and PFS. The subgroup analysis showed that high infiltrated CD8+ T cells in invasive margin were the strongest predictors for ORR (OR, 13.05; 95% CI: 3.79–44.86;  $p < 0.001$ ; heterogeneity,  $p = 0.81$ ) (Fig. 3C; eFigure 4C in the appendix).

### 3.6. Correlation between circulating CD8+ t cells and prognosis

We further investigated the impact of circulating CD8+ T cells from peripheral blood on OS and PFS. We did not find improved OS (HR, 0.93; 95% CI: 0.67–1.29;  $p = 0.67$ ; heterogeneity,  $p = 0.12$ ) and PFS (HR, 0.89; 95% CI: 0.60–1.32;  $p = 0.56$ ; heterogeneity,  $p = 0.10$ ) in patients with high baseline circulating CD8+ T cells, compared to those with low baseline (Fig. 4).

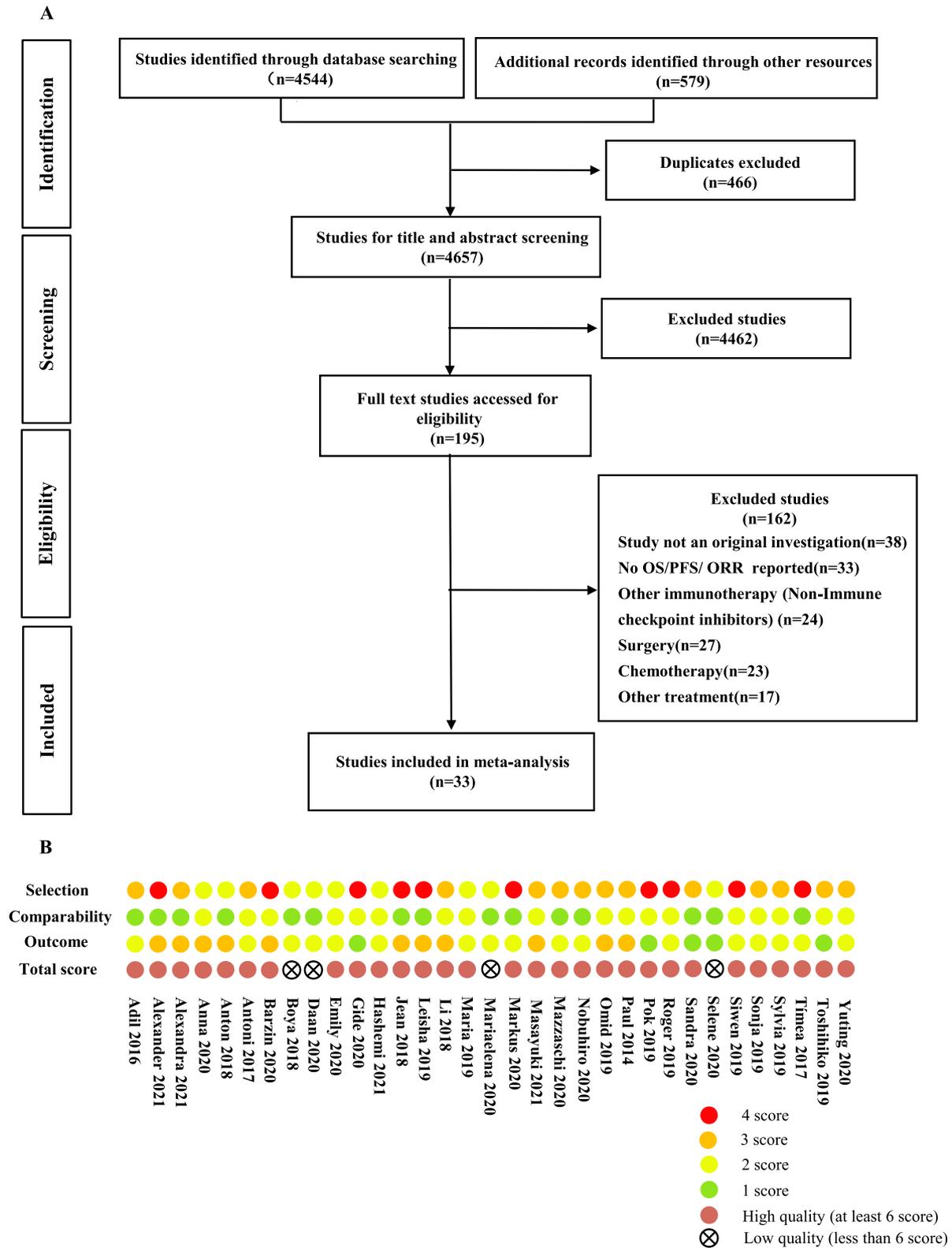
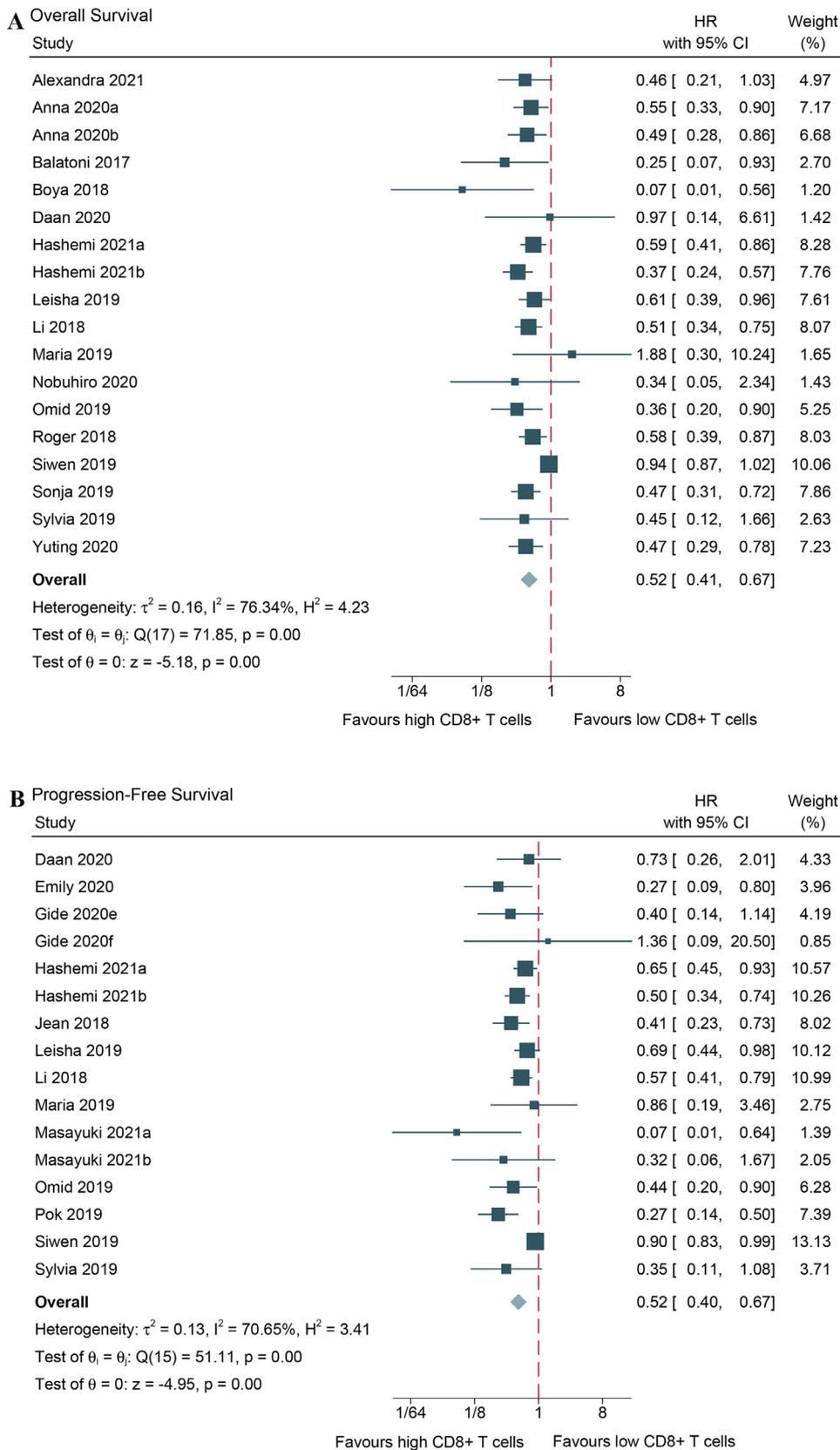


Fig. 1. Flowchart and quality assessment of selecting eligible studies. (A): flowchart of selecting eligible studies. (B): Quality assessment of included studies by NOS.

**Table 1**  
Baseline characteristics of included studies.

Author	Year	Study Type	Number	Country	Age, y	Disease	Treatment	Cutoff Value	Detection	CD8+ T cells Location	Sample Source	Outcome
Adil	2016	Retrospective	40	USA	NA	Melanoma	Pembrolizumab/ Nivolumab	1500 cells/mm2	Flow Cytometric	Invasive Margin and Overall Tumor	Tissue	ORR, PFS, OS
Alexander	2021	Retrospective	17	USA	62(34–77)	Neuroendocrine Neoplasms	Pembrolizumab	Median	Flow Cytometry	Peripheral Blood	Blood	PFS
Alexandra Anna	2021 2020	Retrospective Retrospective	98 139	Canada Netherlands	57(25–86) NA	Solid Tumors NSCLC	Anti-PD-1/ Anti-PD-L1/CTLA-4 Nivolumab	Median Median	NA IHC	NA Intratumor and Stroma	Tissue Tissue	TTP, OS ORR, OS
Anton Anton	2018 2017	Retrospective RCT	56 21	Israel USA	NA 58 (37–89)	NSCLC or Melanoma Melanoma	Pembrolizumab/ Nivolumab Talimogene Laherparepvec plus Pembrolizumab	886 cells/mm2 1000 cells/mm2	IHC NA	Intratumor Intratumor	Tissue Tissue	ORR ORR
Balatoni	2017	Retrospective	30	Hungary	NA	Melanoma	Ipilimumab	Median	IHC	Intratumor	Tissue/ Lymph Node	ORR, OS
Barzin Boya	2020 2018	Retrospective Retrospective	99 18	USA USA	66(29–85) 66(40–80)	NSCLC Bladder Cancer	Anti-PD-L1 Atezolizumab/ Pembrolizumab/ Durvalumab	NA Median	Flow Cytometry IHC	Peripheral Blood NA	Blood Tissue	PFS, DCB DOR, OS
Daan Emily Gide	2020 2020 2020	Retrospective Retrospective Retrospective	30 86 61	Netherlands USA Sydney	64 ± 8.6 NA 67	NSCLC Sarcoma Melanoma	Nivolumab Pembrolizumab Anti-PD-1 / Anti-PD-1 plus Anti-CTLA-4	Median 95 cells/mm2 Median	IHC IF IHC	NA NA Intratumor and Peritumor	Tissue Tissue Tissue	ORR, PFS, OS PFS PFS
Hashemi	2021	Retrospective	141	Netherlands	NA	NSCLC	Nivolumab/ Pembrolizumab	Median	IHC	Intratumor and Stroma	Tissue	PFS, OS
Jean Leisha	2018 2019	Retrospective RCT	85 104	France US and European	66(45–85) 53(29–82)	NSCLC TNBC	Nivolumab Atezolizumab	NA 1.35%	IHC IHC	NA Intratumor and Stroma	Tissue Tissue	PFS ORR, PFS, OS
Li	2018	Retrospective	270	Multiple Regions	66(38–90)	Urothelial Cancer	Nivolumab	Median	IHC	Intratumor and Stroma	Tissue	ORR, PFS, OS
Maria	2019	Retrospective	58	USA	NA	Melanoma	Pembrolizumab/ Nivolumab/ Ipilimumab plus Nivolumab	Median	IHC	Intratumor and Stroma	Tissue	PFS, OS
Mariaelena Markus	2020 2020	Retrospective Retrospective	100 56	Italy Germany	62(28–90) 59 ± 8.6	Melanoma Head and Neck Cancer	Nivolumab Cisplatin/ Docetaxel plus Durvalumab/ Tremelimumab	Median Median	Flow Cytometry IHC	Peripheral Blood Intratumor	Blood Tissue	ORR, OS pCR
Masayuki	2021	Retrospective	13	Japan	62(42–86)	Large cell neuroendocrine carcinoma	Anti-PD-1 therapy	38/mm 2 295/mm 2	IHC	Intratumor and Stroma	Tissue	ORR, PFS
Mazzaschi Nobuhiro	2020 2020	Prospective Retrospective	109 33	Italy Japan	72(41–85) NA	NSCLC NSCLC	Nivolumab/ Pembrolizumab/Atezolizumab Nivolumab	NA NA	Flow Cytometry IHC	Peripheral Blood Intratumor	Blood Tissue	PFS, OS ORR, OS
Omid Paul	2019 2014	Prospective RCT	45 46	USA USA	63(21–83) NA	Melanoma Melanoma	Atezolizumab Pembrolizumab	Median Median	IHC IHC	Intratumor Intratumor and Invasive Margin	Tissue Tissue	ORR, PFS, OS ORR
Pok	2019	Retrospective	94	USA	NA	Melanoma	Pembrolizumab/ Nivolumab/ Ipilimumab+ Nivolumab	NA	IF	Intratumor and Stroma	Tissue	ORR, DCR, PFS
Roger	2018	Retrospective	137	France	58(45–66)	Various Cancer	Anti-PD-1/ Anti-PD-L1	Median	CT Scans, RNA Sequencing	NA	Tissue	ORR, OS
Sandra	2020	Retrospective	88	USA	72.5(33–88)	Merkel Cell Carcinoma	Avelumab	Median	IHC	Invasive Margin	Tissue	ORR, DOR, PFS, OS
Selene Siwen	2020 2019	Prospective Retrospective	74 38	Italy USA	67.6(44–85) 67.5(48–82)	NSCLC NSCLC	Nivolumab Pembrolizumab	Median Median	Flow Cytometry IHC	Peripheral Blood Intratumor	Blood Tissue	ORR, PFS, OS ORR, PFS, OS
Sonja Sylvia	2019 2019	Retrospective RCT	163 33	USA USA	NA 55(32–84)	NSCLC TNBC	Durvalumab Atezolizumab plus Nab-Paclitaxel	Median Median	IHC IHC	NA Intratumor	Tissue Tissue	PFS, OS ORR, PFS, OS
Toshihiko	2019	Retrospective	96	Japan	63(56–68)	Various Solid Tumor	Nivolumab plus Mogamulizumab	Median	IHC	Intratumor and Stroma	Tissue	BOR, TTR, DOR, PFS, OS
Yuting	2020	Retrospective	81	USA	NA	NSCLC	Pembrolizumab/Nivolumab/ Atezolizumab	NA	NA	Intratumor and Stroma	Tissue	OS

Abbreviation: RCT, randomized controlled trial; NA, not available; y, year; TNBC, triple-negative breast cancer; NSCLC, non-small cell lung cancer; OS, overall survival; RR, response rate; ORR, overall response rate; BOR, best overall response; DOR, duration-of-response; DCB, durable clinical benefit; TTR, time-to-response; PFS, progression-free survival; CSS, cancer-specific survival; TTP, time to progression; IHC, immunohistochemistry; IF, immunofluorescence; PD-1, programmed cell death protein 1; PD-L1, programmed death–ligand 1; CTLA-4, cytotoxic T-lymphocyte–associated antigen 4.



**Fig. 2.** Forest plot of HR and OR of high CD8+ TILs versus low CD8+ TILs for OS, PFS and ORR in various cancer patients treated with ICIs. (A): pooled HR of OS for patients treated with ICIs. (B): pooled HR of PFS for patients treated with ICIs. (C): pooled OR of ORR for patients treated with ICIs.

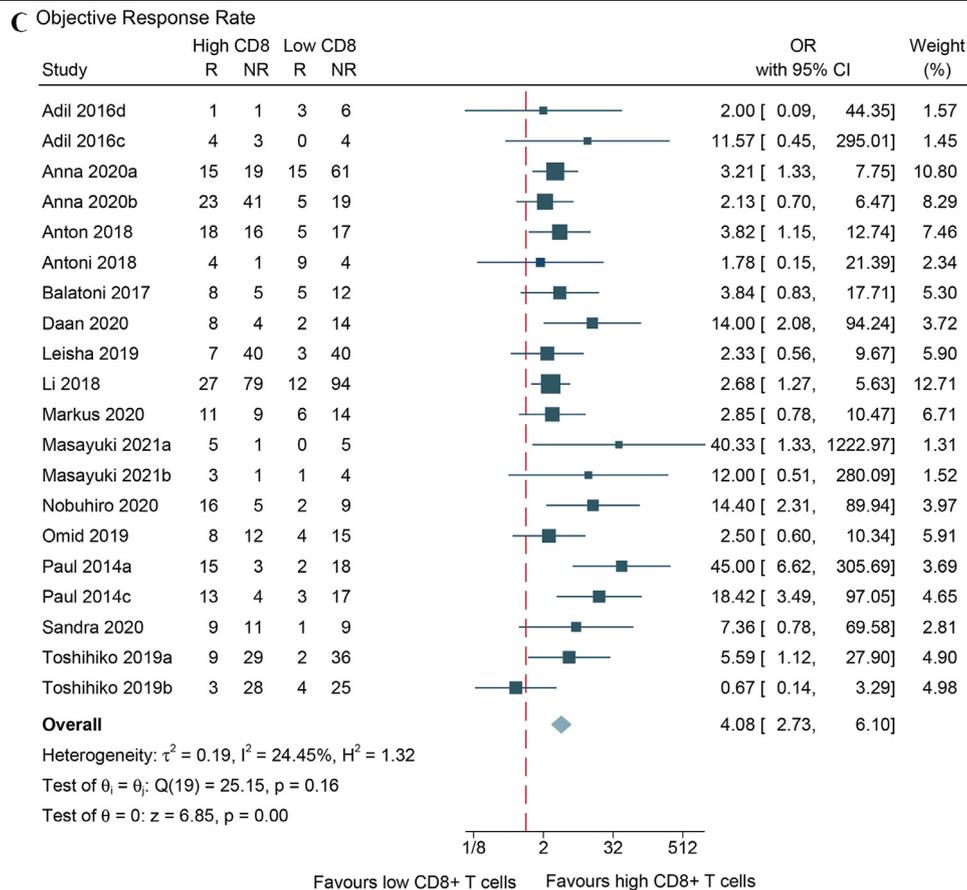


Fig. 2. Continued.

### 3.7. Publication bias assessment

The funnel plot and Egger's test result revealed that publication bias existed in studies of OS (Egger's test,  $p < 0.001$ ; eFigure 5A in the appendix) and PFS (Egger's test,  $p < 0.001$ ; eFigure 5B in the appendix). The funnel plot for the ORR revealed no asymmetry (Egger's test,  $p = 0.114$ ; eFigure 5C in the appendix), indicating no obvious publication bias regarding ORR.

### 3.8. Sensitivity analysis

To evaluate the robustness of the combined outcomes, we carried out sensitivity analyses by omitting specific studies or excluding the low quality studies. The result showed that the meta-analysis had low sensitivity and overall estimates remained consistent across these analyses (eFigure 6, eFigure 7 in the appendix).

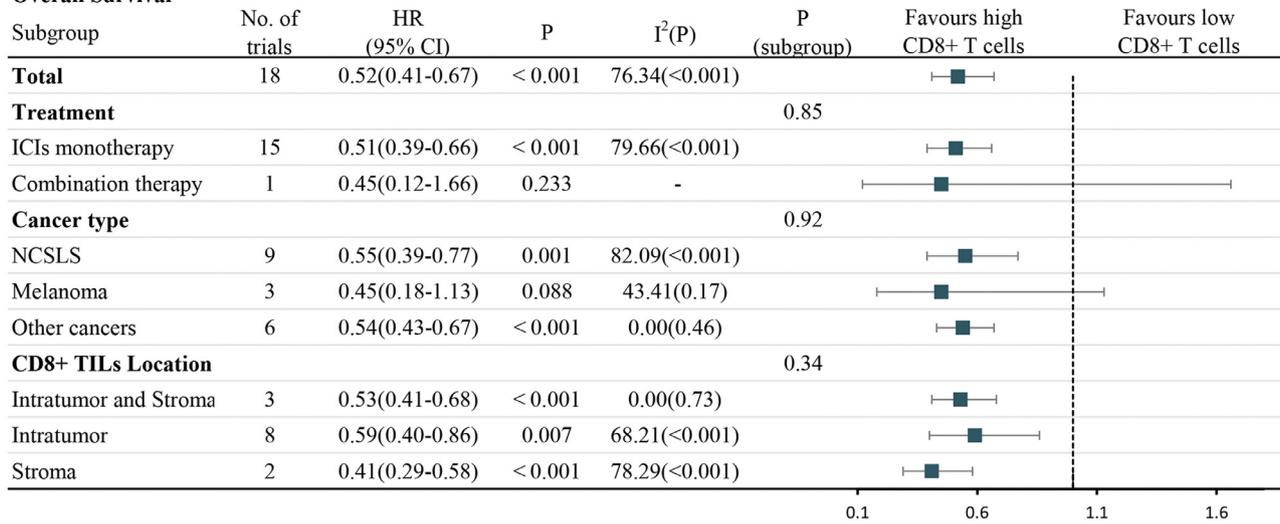
## 4. Discussion

Tumor regression induced by ICIs is influenced by factors related to the tumor microenvironment [36,47]. In recent years, enormous efforts have been made in the assessment of the predictive value of different tumor-infiltrating immune cell subsets in patients with ICIs [48]. In this study, we found that the CD8+ TILs was a significant biomarker to predict the efficacy of ICIs across different cancers, in either single-agent ICIs or combination with other therapies. We also highlighted that high CD8+ TILs within stroma and invasive margin compartment had a better outcome than those in intra-tumor compartment. No expectation of longer survival was observed for patients with high baseline circulating CD8+ T cells.

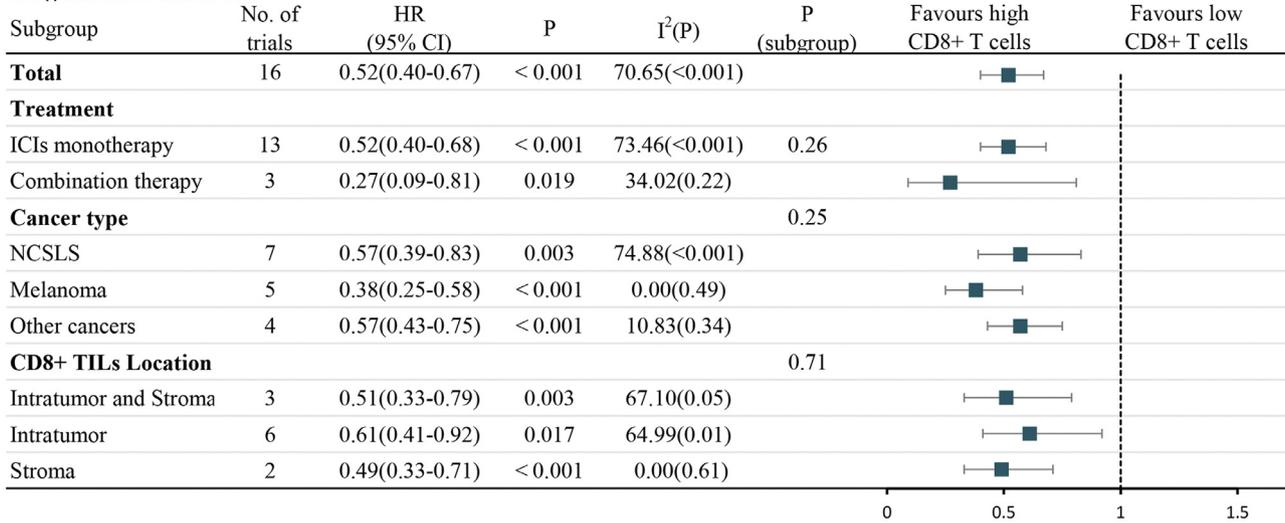
Our result was consistent with the previous analyses of 15 tumor-infiltrating immune cell subtypes in 17 cancers of all stage; CD8+ TILs was the strongest predictive biomarker in clinical benefit for cancer patients [48]. CD8+ TIL was regarded as a key player in killing cancer cells via releasing cytotoxic molecules and cytokines, but its function could be spoiled by the signaling produced by PD-1/PD-L1 axis [49]. ICIs could significantly recruit tumor-infiltrating tumor-specific CD8+ T cells and reverses the exhausted T cell phenotype, which is critical for restored immune surveillance and tumor killing activity of CD8+ T cells [49,50], uncovering that the pre-existing antitumor adaptive immune reaction may be of great significance for patient survival.<sup>51</sup>

ICIs combination therapy has been a trend in cancer treatment. However, prognostic biomarkers related to ICIs combination therapy in cancer patients are still lacking since most studies are focused on the biomarker in patients treated with ICIs mono therapy. Besides, The combination of drugs, for example, chemotherapy, can modify the tumor microenvironment and potentially affect the composition of immune cells, which make conventional biomarkers, such as PD-L1, TMB, unable to predict the efficacy of ICIs plus chemotherapy [52,53]. Moreover, in IMpassion 130 trial, atezolizumab in combination with nab-paclitaxel showed a benefit in PFS and OS in the metastatic triple-negative breast cancer (TNBC) population, however, the IMpassion 131 trial showed a discrepant finding, even in PDL-1 positive population [54,55]. There is growing concern that the suboptimal assay used in these trials (SP142 PDL1) is partly the reason on the discrepancies observed between these trials [56]. In the biomarker evaluation of the IMpassion130 study [57], high tumor-infiltrating CD8+ T cells was associated with better prognosis in patients treated with atezolizumab plus nab-paclitaxel (Median PFS: 7.4 months vs. 5.6 months; Median OS: 22.6 months vs. 16.3 months), regardless of PD-

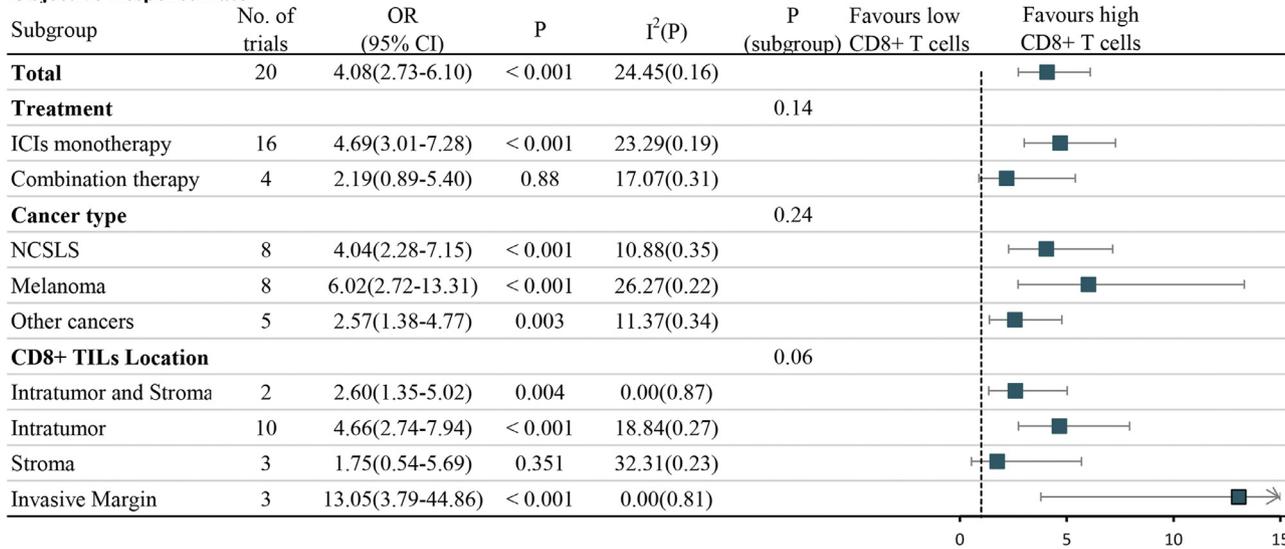
**A Overall Survival**



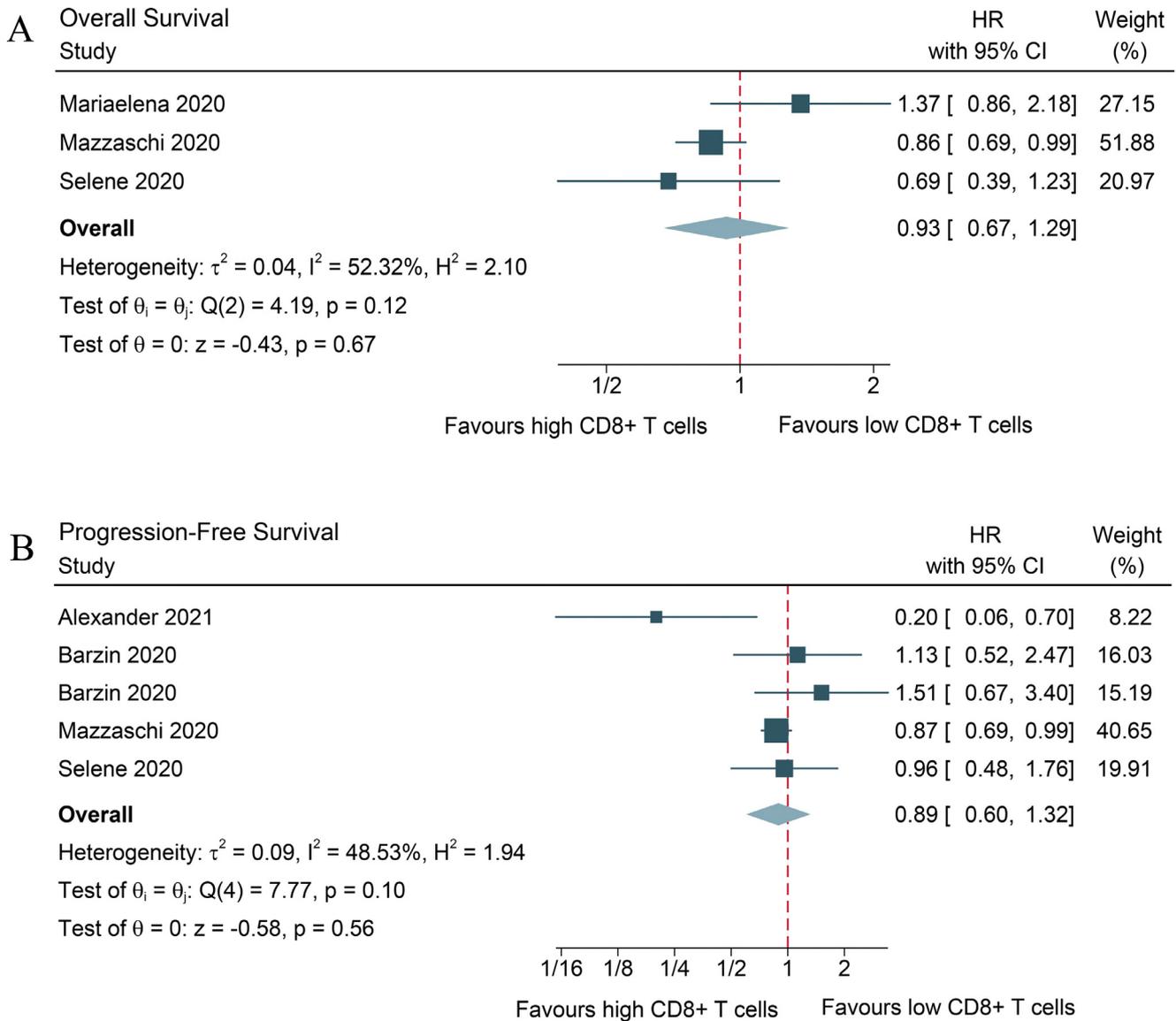
**B Progression-Free Survival**



**C Objective Response Rate**



**Fig. 3.** Subgroup analyses of OS, PFS, and ORR with regard to different treatment types, cancer types, CD8+ T cells location. (A): Forest plot of HR in subgroup-analyses comparing OS in patients who received ICIs. (B): Forest plot of HR in subgroup-analyses comparing PFS in patients who received ICIs. (C): Forest plot of OR in subgroup-analyses comparing ORR in patients who received ICIs.



**Fig. 4.** Forest plot HR of OS and PFS of high CD8+ T cells versus low CD8+ T cells from peripheral blood in various cancer patients treated with ICIs. (A): pooled HR of OS for patients treated with ICIs. (B): pooled HR of PFS for patients treated with ICIs.

L1 status. In addition, in the KEYNOTE-086 trial [58,59], a statistically significant positive linear association between expression of the tissue-resident memory T cells (one of the subtypes of memory T cells) gene signature and response rate were observed in more than 200 patients with advanced-stage TNBC receiving pembrolizumab. We proposed that CD8+ TILs may be helpful to explain the discrepant findings between Impassion130 and Impassion131. The incorporation of memory T cells evaluations into traditional TIL quantification methods might further inform decisions regarding the selection and stratification of cancer patients in future.

Compared to high CD8+ T cells infiltration in total tumor tissue or intra-tumor, a potential trend for better efficacy was presented in patients with high CD8+ T cells infiltration in stroma or invasive margin. The important role of CD8+ T cells in stroma or invasive margin has already been emphasized in postoperative cancer patients, such as colorectal cancer [51,60], tongue squamous cell carcinoma [61] and so on. For patients treated with ICIs, Paul et al. firstly demonstrated that invasive marginal CD8+ TILs worked as a better predictive parameter than the intra-tumoral CD8+ TILs [36], but little information was provided in the underlying mechanisms regarding

the spatial distribution and prognosis. Other studies may provide some explanations for this phenomenon. Some experts find that the invasive margin is a critical area for stimulating angiogenesis and lymphangiogenesis in tumor, which contributes to tumor invasion and metastasis [62]. CD8+ T cells at the invasive margin are negatively related to the depth of invasion and vascular invasion [62]. CD8+ T cells infiltration at the invasive margin, compared with that in the inner part of tumor, is more effective against tumor development. Moreover, despite their cytotoxic effect in tumor, prolonged exposure of CD8+ TILs in tumor bed may lead to intra-tumoral CD8+ T cell exhaustion, which is mediated by tumor cell PD-L1 expression [63]. Hence, after stimulatory immunotherapy by ICIs, CD8+ TILs at the invasive margin performed higher degrees of anticancer activity as compared to intra-tumoral CD8+infiltration.

The above findings may be limited by the small number of studies and that the conclusion about predictive value of stromal CD8+ T cells in ICIs should be viewed with caution. Colt et al. have drawn a contrary conclusion that infiltration of CD8+ T cells into cancer islands was more significantly associated with the relapse-free survival than CD8+ T cell infiltration into either total tumor or stroma, while the

result was not related to ICIs therapy [64]. In the stroma, CD8+TILs show a strong positive association with positive PD-L1 expression [65,66]. Low stromal CD8+ T cells infiltration was positively correlated to an increased incidence of angiolymphatic invasion [67]. These may partially explain the relationship between high stromal CD8+ T cells and clinical benefit in patients treated with ICIs. Our finding preliminarily confirmed the anti-cancer effect of CD8+ TILs, regardless of the location. However, considering the complex interactions between tumor cells and TILs in tumor immune microenvironments, which CD8+ infiltrating location (intra-tumor, stroma and invasive margin) has more effective activity in patients treated with ICIs, it needs to be further research.

There are many kinds of lymphocytes in the peripheral blood, and their functions are complex. In our result, although circulating CD8+ T cells produced the modest efficacy in patients with ICIs treatment, CD8+ T cells sub-population, such as PD-1+CD8+ T cells, TCF7+CD8+ T cells, CD8+ memory cells, and so on, are positive prognostic biomarkers for survival [10,33,68–70]. Under physiological conditions and chronic infection, effector memory CD8+ T cell subsets with high levels of cytolytic molecules expression selectively remained in the intravascular circulation, instead of migration to tissue [71]. Whether there is greater homing of CD8+ T cells to tumor deposits and play an anticancer role in patients after ICIs treatments, it is still unclear.

Our results show that cancer patients with high CD8+ TILs have longer OS and PFS, suggesting that the presence of high CD8+ TILs is a good prognostic factor for patients treated with ICIs. At the same time, we also found that cancer patients with high CD8+ TILs had a higher ORR, suggesting that the presence of high CD8+ TILs is also a potential predictive factor of favorable outcome for patients treated with ICIs. To explore the predictive effect of CD8+ TILs, we should further explore the predictive value of CD8+ TILs among different treatment group (for example, ICI group vs. chemotherapy group). In addition, clinical trial designs should be more carefully conducted in order to distinguish the prognostic effect from the predictive effect. Our data enforce the increasing relevance of the evaluation of immune cells in clinical trial and daily practice, according to established guidelines [72]. As evidence indicates that CD8+ TILs reflect the stromal TILs ([www.tilsinbreastcancer.org](http://www.tilsinbreastcancer.org)). Since both reflect the same population [73], both can help identifying patients that may benefit to immunotherapy, as demonstrated in several phase 3 clinical trials (Impassion130 and KN119). Considering the increasing criticisms on PDL1-assays, CD8+ TILs, as an alternative to PDL1-assays, may have more clear evidence of predicting benefit to immunotherapy.

There are several limitations in our study. First, although we performed the subgroup analysis and sensitivity analysis, the heterogeneity was not significantly decreased. Second, some articles only presented ORR without OS and PFS. Few studies focused on the role of CD8+ T cells infiltration in the stroma and invasive margin. Most patients were treated with ICIs mono therapy, and ICIs combination therapy was less common. Third, not all cancer types were included in our meta-analysis, especially advanced clear cell renal cell carcinoma, which is known that CD8+ infiltration is not predictive of response to immunotherapy. This may lead to selection bias and the result should be viewed with caution. Fourth, the CD8+ cutoff value in this analysis is not uniform, which needs further studies to clarify. A Bayesian approach may be able to determine an initial cut-off in CD8+ expression based on prior information from other trials [74]. According to Bellini-trial, different categories of TILs/CD8-scores could also be used to identify in which category the best responders can be found, as this would be informative for finding an appropriate cut-off. It is significant to further explore the linear association between CD8+ TILs density and response rate, when CD8+ TILs density works as a continuous variable. Fifth, the stromal components are not clearly defined in the original article and may or may not consist of invasive margin components, leading to an inconsistent

conclusion on different components. Despite these limitations, this meta-analysis contributes to our understanding of the predictive role of CD8+ TILs in immunotherapy.

In conclusion, the result suggested that high CD8+ TILs were associated with favorable outcomes in cancer patients with ICIs therapy, regardless of ICIs-treatment regime, cancer types and CD8+ T cells locations.

## Contributors

FL, CCL, XYC, ZHX contributed to data acquisition, data interpretation, and statistical analysis and drafting of the manuscript. LQZ, BC, SX and JFL contributed to data acquisition, data interpretation, and statistical analysis. WHL and HJX contributed to the study design, data acquisition, data interpretation and revision of the manuscript. All the other authors (RZ, ZXC and ZWY) contributed to data interpretation and critical revision of the manuscript. All authors have final approval of the submitted manuscript and reached agreement to be accountable for all aspects of the work.

## Data sharing

This manuscript makes use of publicly available data from published studies; therefore, no original data are available for sharing.

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## Declaration of Competing Interest

No potential conflicts of interest were disclosed.

## Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.eclinm.2021.101134.

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