Prognostic Significance of CD4+ Tumour Infiltrating Lymphocytes in Patients with Non-Small Cell Lung Cancer Receiving PD-1/PD-L1 Inhibitors Therapy

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ABSTRACT

CD4+ tumour infiltrating lymphocytes (TILs) have been found to produce a marked effect in anti-tumour immunity. In the present study, the authors explored the predictive value of CD4+ TILs in patients with non-small cell lung cancer (NSCLC) receiving PD-1/PD-L1 inhibitors therapy. The authors searched Cochrane, Embase, PubMed, and the Web of Science with a November 2023 deadline. This study followed the requirements of the preferred reporting items for systematic reviews and meta-analyses (PRISMA). The data were analysed by Stata MP17.0 software. Endpoints included objective response rate (ORR), progression-free survival (PFS), and overall survival (OS). In total, 13 studies ultimately met the inclusion criteria. Findings showed high levels of CD4+ TILs in tumour tissue were correlated with better ORR (OR = 1.78, 95% CI: 1.15-2.76, p = 0.010) in NSCLC patients, rather than PFS (HR = 0.82, 95% CI: 0.65-1.05, p = 0.11) and OS (HR = 0.86, 95% CI: 0.69-1.09, p = 0.217). In addition, high levels of peripheral blood CD4+ T cells correlated with better PFS (HR = 0.66, 95% CI: 0.46-0.94, p = 0.02), rather than OS (HR = 0.90, 95% CI: 0.69-1.19, p = 0.461). The results demonstrated that high CD4+ TILs in tumour tissue can predict better ORR for NSCLC patients receiving PD-1/PD-L1 inhibitors, and high peripheral blood CD4+ T cells can predict better PFS.

Key Words: *CD4-positive T-lymphocytes, Immune checkpoint inhibitors, Non-small cell lung cancer, Peripheral blood, Prognosis, Tumour-infiltrating*.

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INTRODUCTION

Lung cancer incidence and mortality have declined in recent decades, but it remains the main reason for death from tumours globally.[1] Non-small cell lung cancer (NSCLC) accounts for about 85% of lung cancer subtypes.[2] Currently, surgical intervention is the primary clinical treatment choice for early-stage NSCLC patients.[3] However, more than 70% of patients with NSCLC are diagnosed as suffering from an intermediate to advanced stage on their first visit to the doctor.[4] Even with various clinical treatment strategies, such as radiotherapy and chemotherapy,[5] treatment outcomes remain unsatisfactory.[6,7]

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Since the 2010s, the use of immune checkpoint inhibitors (ICIs) has transformed the treatment of a number of malignant tumours, including NSCLC. Among them, programmed cell death protein 1 (PD-1) inhibitors and programmed cell death ligand 1 (PD-L1) inhibitors are more common in clinical practice. Indeed, cancer patients respond differently to ICIs, and clinical studies have reported that many cancer patients cannot benefit from ICIs and even suffer severe life-threatening immune toxicity.[8-10] Therefore, there is an urgent need to find predictive biomarkers that reflect the good or bad efficacy of immunotherapy to further enhance the therapeutic efficacy of ICIs.

PD-L1 immunohistochemical test can be used as a concurrent or supplemental diagnosis for patients with NSCLC receiving ICI treatment.[11] Tumour mutational load (TML) has also been shown to be a valid predictor. However, there is still much controversy regarding the use of these two substances as biomarkers.[12-14] However, tumour infiltrating lymphocytes (TILs) in tumour tissue or peripheral blood are gradually gaining attention. ICIs can overcome T cell dysfunction and exhaustion caused by the regulation of transcription and translation from

different cell populations in the tumour microenvironment (TME).[15] In solid tumours, the main ingredient of the TIL compartment is T lymphocytes.[16] CD8+ TILs have been shown to be the key determinants of treatment response to ICIs, as they play a direct role in tumour cell destruction,[11] and few studies have noted a significant correlation between high CD8+ TILs in tumour tissue and better prognosis in patients receiving ICIs.[17,18] However, only a few studies preliminarily explored the clinical significance of CD4+ TILs as predictive markers of immunotherapy efficacy in NSCLC patients. However, the findings of these small sample size studies were inconsistent, which limited their clinical application.

Here, the authors retrieved data from recent clinical studies and analysed them integrally to elucidate the prognostic significance of CD4+ TILs in NSCLC patients receiving PD-1/PD-L1 inhibitors, and discussed the role of circulating CD4+ T cells from peripheral blood.

METHODOLOGY

This study was conducted in accordance with PRISMA guidelines.[19,20] It was registered in the PROSPERO (Number: CRD 42023409436). Cochrane, Embase, PubMed, and Web of Science were searched from the start of the build till November 2023. Authors used Medical Subject Headings (MeSH) words (Lymphocytes, Tumour-infiltrating; CD4-positive T-lymphocytes; Carcinoma, Non-small-cell lung; Immune checkpoint inhibitors) as well as their matching entry terms, with the MeSH words and corresponding terms connected by 'OR' and each group of topics connected by 'AND'.

The inclusion criteria after searching the literature were as follows. Patient must have a diagnosis of primary NSCLC confirmed through cytology or pathology. The type of study considered must be observational or interventional. The intervention method should involve PD-1/PD-1-L1 inhibitors, including or excluding other therapies and all lines of therapy. The control group consisted of high and low CD4+TILs. The outcome included complete results (ORR, PFS, OS) and their statistical values, or sufficient resources to extract the above information. The exclusion criteria were metastatic or recurrent tumour; animal experiments, reviews, meta-analyses, or case reports; other ICIs, such as CTLA-4 inhibitors; incomplete data or inability to extract relevant data; and quality deficiencies.

For the data extracted, two researchers worked independently and any divergences were solved through deliberation with a third fellow. The extracted information contained: Basic characteristics, patient characteristics, information on CD4+ TILs, and endpoints. Objective response rate (ORR) referred to the sum of complete remission (CR) and partial remission (PR) in patients after treatment, as assessed by the Response Evaluation Criteria in Solid Tumours (RECIST) or immune-related response criteria (irRC). Progression-free survival (PFS) referred to the time from the beginning of a patient's treatment to tumour progression or death because of any reason. Overall survival (OS) referred to the time from the beginning of a patient's treatment to death due to any reason.

Two researchers used the Newcastle-Ottawa Scale (NOS) to separately assess the quality of the included studies,[21] and any divergences were solved through deliberation with a third fellow. The tool has a total score of 9, with scores of 7-9/4-6/0-3 corresponding to high/medium/low-quality literature, respectively.

The pooled data were analysed using Stata version 17.0 MP. The combined odds ratio (OR) and its 95% confidence interval (CI) were used to assess the effect size of ORR, and the authors used the random-effects DerSiminian-Laird model to calculate the estimates. The combined hazard ratio (HR) and its 95% CI were utilised to compute the effect size of OS and PFS, and the authors calculated the estimates by using the random-effects inverse variance weighted model. For the heterogeneity among the studies, authors assessed I^2 Statistic. If the I^2 statistic was >50%, significant heterogeneity between studies may exist. To explore significant influences on heterogeneity, authors performed subgroup analyses. The robustness of the combined results was assessed through a sensitivity analysis based on the risk of bias. It was done by excluding the results of any of the included studies to observe whether the combined OR or HRs of the remaining studies were within 95% CI of the initial combined results. The funnel plot may not be objective to assess publication bias due to the number of included studies, so the authors used Egger's algorithm for further quantitative analysis and a p-value of <0.05 suggests publication bias. OR and 95% CI were used as measures of dichotomous experimental effects, and the composite effect values were statistically tested. OR is the ratio of the statistic of a low level of CD4+ TILs to a high level of CD4+ TILs, and OR>1 suggests a higher propensity to die in patients with lower levels of CD4+ TILs. When included studies had both univariate and multivariate analyses of effect sizes, the authors extracted data from multivariate analyses. In individual studies, where data are not straightforward, if the article does not provide certain HR but instead provides Kaplan-Meier, authors used Engauge Digitisation software (Sourced from: https://markummitchell.github.io/engauge-digitizer/) to extract data from Kaplan-Meier curves and digitise and estimated the single variable HR and 95% CI through the Excel macro file provided by their team.[22] The authors also utilised the GetData Graph Digitiser software (sourced from: https://www.getdata-graph-digitizer.com/) to roughly estimate the HR of studies that only provided forest plots. For all pooled analyses, $p < 0.05$ suggests a statistically significant difference.

RESULTS

In total, 13 studies ultimately fully met the eligibility criteria (Figure 1).[23-35] The basic information and quality assessment of the included studies are presented in Table I and II, respectively. Of these 13 studies, seven were assessed as high quality and the rest were assessed as medium quality. It demonstrated the relatively high overall quality of the included studies.

Table I: General features of included studies.

Author	Year	Country	Study type	Number	Age	Histology subtype	Medicine	Sample source	CD4+ TILs Location	Detection method	CD4+ TILs cut-off value	Outcomes	Study quality
Du *et al.*[23]	2023	China	Retrospective	146	64 (56-69)	NSCLC	Pembrolizumab/toripalimab/camrelizumab/sintilimab/tislelizumab	Blood	Peripheral blood	Flow cytometry	39.95	OS, PFS	6
Lieskovan *et al.*[24]	2019	USA	Retrospective	38	67.5 (48-82)	NSCLC	Pembrolizumab	Biopsies/resected samples	Intratumour	Immunohistochemistry	Median	OS, PFS, ORR	8
Kaira *et al.*[25]	2023	Japan	Retrospective	107	NA	NSCLC	Pembrolizumab	Biopsies/resected samples	Intratumour and stroma	Immunohistochemistry	10 and 20	OS, PFS, ORR	8
Koh *et al.*[26]	2022	Korea	Retrospective	59	67 (32-81)	NSCLC	Nivolumab/pembrolizumab/atezolizumab	NA	NA	Immunohistochemistry	Median	ORR	7
Li *et al.*[27]	2021	China	Retrospective	28	65 (NA)	NSCLC	Programmed cell death protein 1 inhibitors	Blood	Peripheral blood	Flow cytometry	Median	PFS, ORR	6
De Rodas *et al.*[28]	2022	USA	Retrospective	179	65 (NA)	NSCLC	Nivolumab/pembrolizumab/atezolizumab	Resected samples	Intratumour and stroma	Immunohistochemistry	446.7 cell/mm^2	OS, PFS	7
Mazzaschi *et al.*[29]	2020	Italy	Prospective	109	72 (41-85)	NSCLC	Nivolumab/pembrolizumab/atezolizumab	Blood	Peripheral blood	Flow cytometry	NA	OS, PFS	6
Niemeijer *et al.*[30]	2020	Netherlands	Retrospective	139	NA	NSCLC	Nivolumab	Biopsies	Intratumour and stroma	Immunohistochemistry	Median	OS, ORR	7
Ottonello *et al.*[31]	2020	Italy	Prospective	74	67.6 (44-85)	NSCLC	Nivolumab	Blood	Peripheral blood	Flow cytometry	Median	OS, PFS, ORR	5
Uryvaev *et al.*[32]	2018	Israel	Retrospective	26	62.5 (46-82)	NSCLC	Nivolumab	Biopsies/resected samples	Intratumour	Immunohistochemistry	300 cell/mm^2	ORR	6
Wu *et al.*[33]	2022	China	Retrospective	109	65 (36-85)	NSCLC	Pembrolizumab/nivolumab/durvalumab/sintilimab/toripalimab/atelizumab	Blood	Peripheral blood	Flow cytometry	266 M/L	OS, PFS	7
Yan *et al.*[34]	2022	China	Retrospective	125	NA	NSCLC	Pembrolizumab/nivolumab/camrelizumab/sintilimab	Blood	Peripheral blood	Flow cytometry	Median	PFS	7
Zugazagoitia *et al.*[35]	2020	USA	Retrospective	53	NA	NSCLC	Nivolumab/pembrolizumab/atezolizumab	Microarrays	Stroma	Immunoflu orescence	Median	OS, PFS	5

NA, Not available; NSCLC, Non-small cell lung cancer; ORR, Overall response rate; OS, Overall survival; PFS, Progression-free survival.

Table II: Quality evaluation of included studies.

Study	Selection				Compatibility	Outcomes			Total Nos Scores
	Representativeness of exposed	Selection of non-exposed	Ascertain-ment of exposure	Demonstration that outcome of interest was not present at the start of the study	Comparability of cohorts on basis of the design or analysis	Assessment of outcome	Was follow-up long enough for outcomes to occur	Adequacy of follow-up of cohorts	
Du *et al.*[23]		√	√		√	√	√		6
Lieskovan *et al.*[24]	√	√	√	√	√√	√	√		8
Kaira *et al.*[25]	√	√	√		√√	√	√	√	8
Koh *et al.*[26]	√	√	√		√√	√	√		7
Li *et al.*[27]	√	√	√		√	√	√		6
Lopez *et al.*[28]	√	√	√		√√	√	√		7
Mazzaschi *et al.*[29]	√	√	√		√	√	√		6
Niemeijer *et al.*[30]	√	√	√		√√	√	√		7
Ottonello *et al.*[31]	√	√			√	√	√		5
Uryvaev *et al.*[32]	√	√			√	√	√	√	6
Wu *et al.*[33]	√	√	√		√√	√	√		7
Yan *et al.*[34]	√	√			√√	√	√	√	7
Zugazagoitia *et al.*[35]	√	√			√	√	√		5

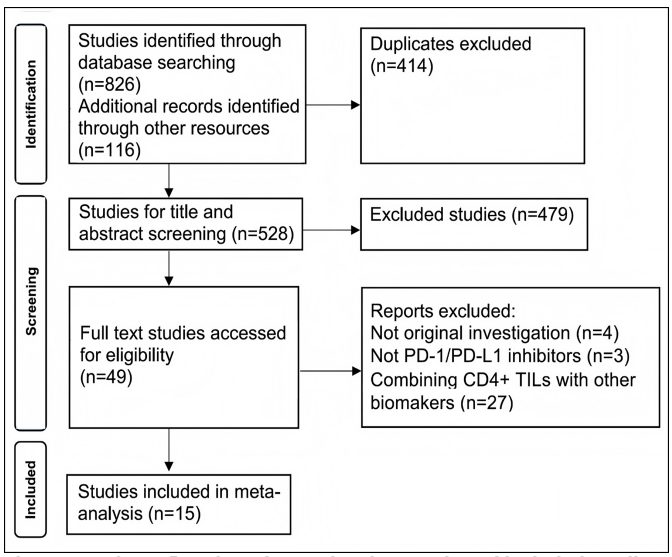

Figure 1: Prisma flowchart for retrieval screening of included studies.

The pooled data showed that high CD4+ TILs in tumour tissue significantly improved ORR but not PFS or OS in NSCLC patients receiving PD-1/PD-L1 inhibitors. The pooled results of five studies showed the ORR with high CD4+ TILs was markedly higher than low CD4+ TILs patients (OR = 1.78, 95% CI: 1.15-2.76, p = 0.010, Figure 2A). The pooled results of four studies reported that high CD4+ TILs in these patients trended towards improved PFS (HR = 0.82, 95% CI: 0.65-1.05, p = 0.11), although not statistically significant (Figure 2B). The pooled results of six studies reported that high CD4+ TILs in these patients trended towards improved OS (HR = 0.89, 95% CI: 0.72-1.11, p = 0.294), although not statistically significant (Figure 2C). Analyses of PFS were highly heterogeneous (I^2 = 57.3%, p = 0.039), instead of ORR (I^2 = 0%, p = 0.638) and OS (I^2 = 48.6%, p = 0.070). Subgroup analysis revealed no significant correlation between CD4+ TILs and combined HR for PFS, regardless of whether they were located within the tumour or in the stroma (Intratumour: HR = 0.96, 95% CI: 0.91-1.02, p = 0.795; Stroma: HR = 0.49, 95% CI: 0.24-1.04, p = 0.062, Figure 2B), and similar conditions were found in the pooled HR of OS and CD4+ TILs (Intratumour: HR = 0.98, 95% CI: 0.93-1.03, p = 0.686; Stroma: HR = 0.65, 95% CI: 0.39-1.08, p = 0.062, Figure 2C).

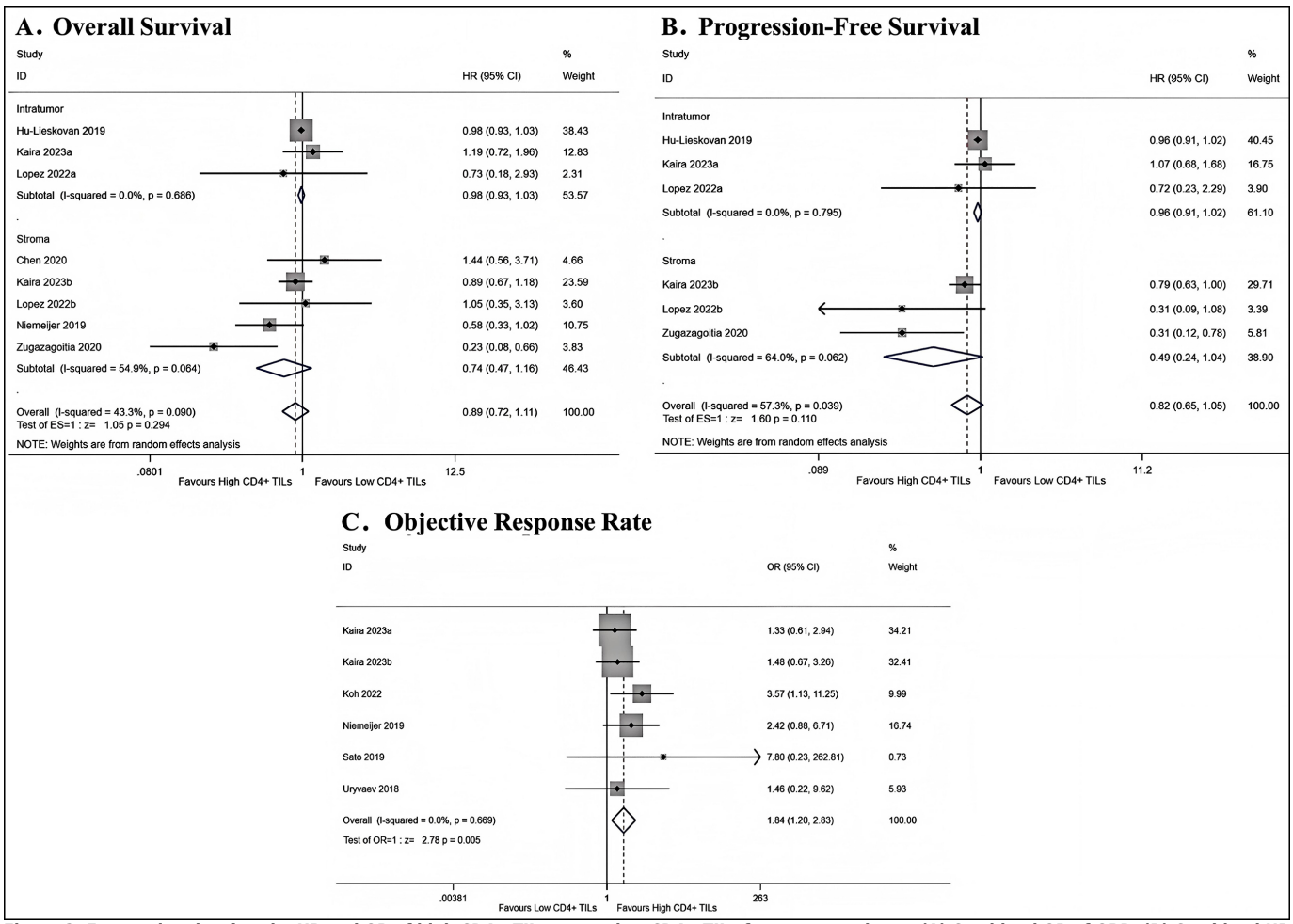

Figure 2: Forest plot showing the HR and OR of high CD4+ TILs *versus* low CD4+ TILs from tumour tissue. (A) Combined OR of ORR. (B) Combined HR of PFS. (C) Combined HR of OS.

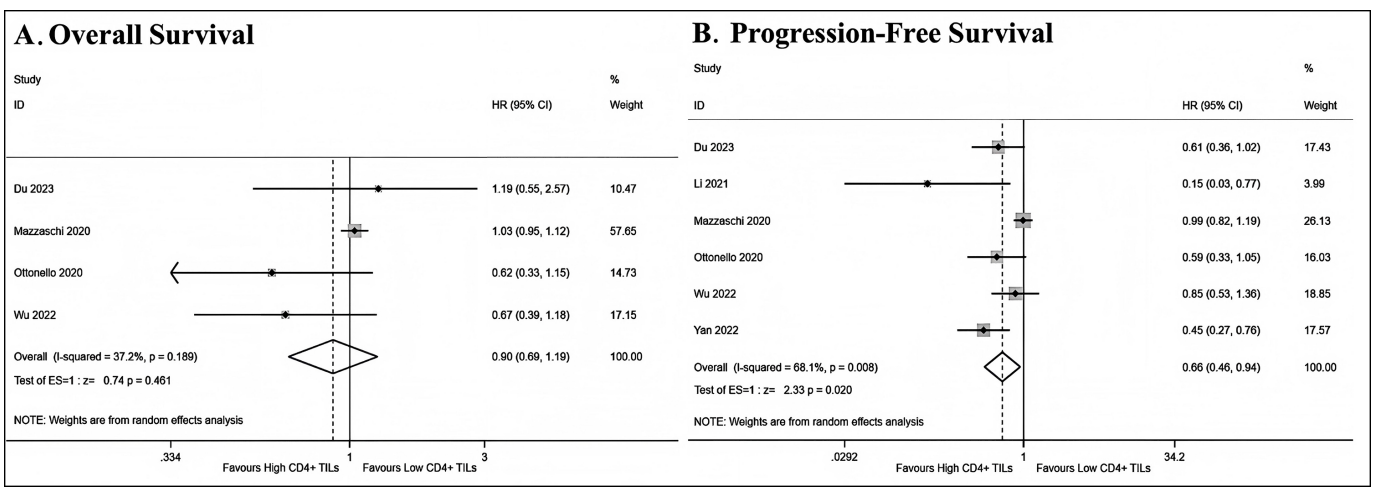

Figure 3: Forest plot showing the HR of high CD4+ T cells versus low CD4+ T cells from peripheral blood. (A) Combined HR of PFS. (B) Combined HR of OS.

The authors further investigated the prognostic significance of CD4+ T cells from peripheral blood. The pooled results of six studies showed the PFS with high CD4+ T cells was markedly higher than low CD4+ T cell patients (HR = 0.66; 95% CI: 0.46-0.94; p = 0.02) (Figure 3A). The pooled results of four studies reported that high CD4+ T cells in these patients trended towards improved OS (HR = 0.90; 95% CI: 0.69-1.19; p = 0.461), although not statistically significant (Figure 3B). Analyses of PFS were highly heterogeneous (I^2 = 68.1%, p = 0.08) instead of OS (I^2 = 37.2%, p = 0.189).

When studying CD4+ TILs in tumour tissue, the funnel plots of pooled ORR, PFS, and OS were relatively symmetric (Figure 4 A-C), and Egger's test revealed that ORR (p = 0.516), PFS (p = 0.090), and OS (p = 0.191). When studying CD4+ T cells from peripheral blood, the funnel plot of pooled PFS was asymmetric (Egger's test, p = 0.010, Figure 4D), indicating the presence of publication bias. In contrast, the funnel plot of pooled OS was relatively symmetric (Egger's test, p = 0.317, Figure 4E).

Sensitivity analysis showed the relative stability of these pooled results (Figure 5). When studying CD4+ TILs in tumour tissue, the heterogeneity of the combined HR for PFS (I^2 = 57.3%) was from Lieskovan *et al*.[24]

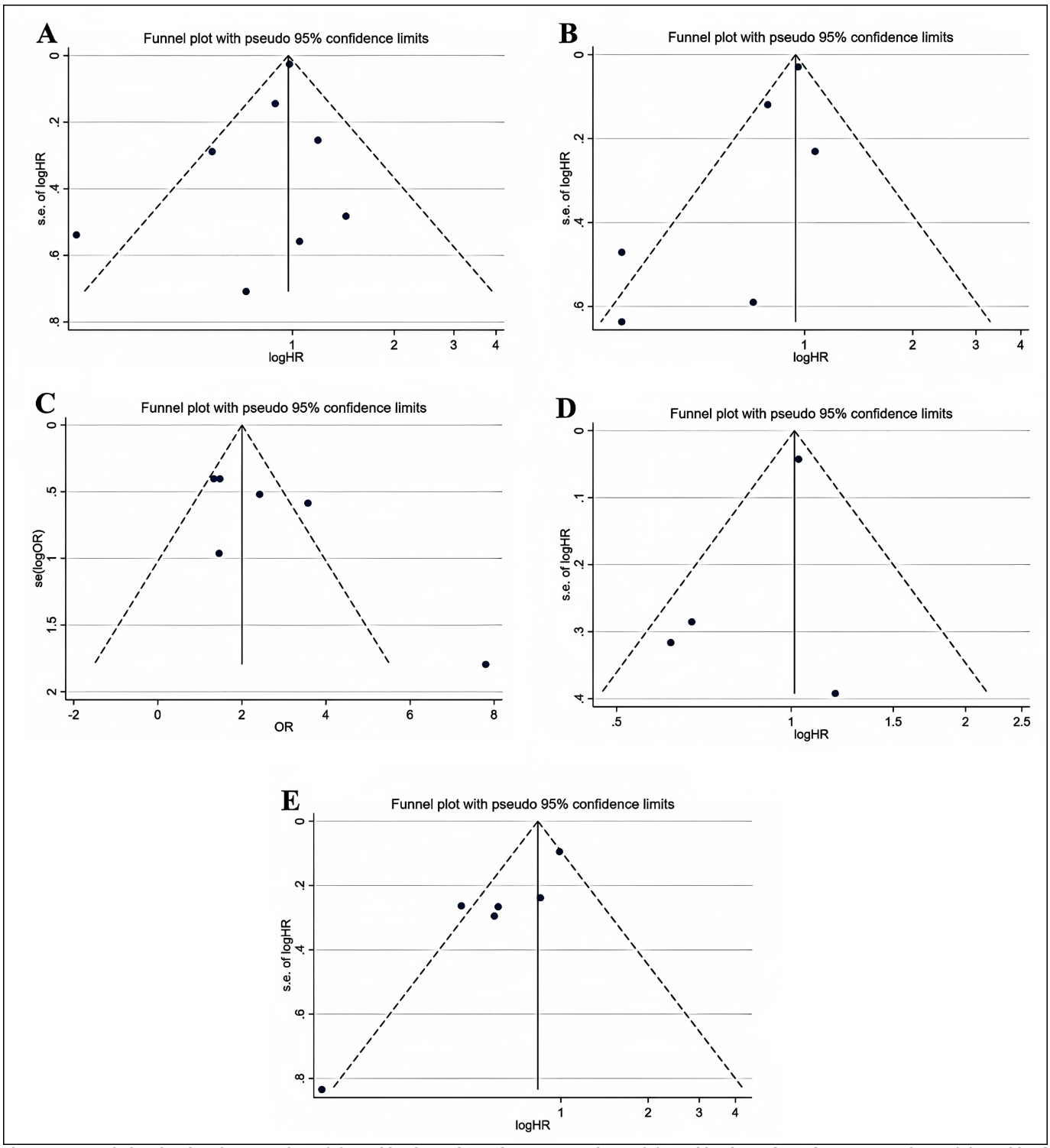

Figure 4: Funnel plot showing the HR and OR. (A) Combined OR of ORR from tumour tissue. (B) Combined HR of PFS from tumour tissue. (C) Combined HR of OS from tumour tissue. (D) Combined HR of PFS from peripheral blood. (E) Combined HR of OS from peripheral blood.

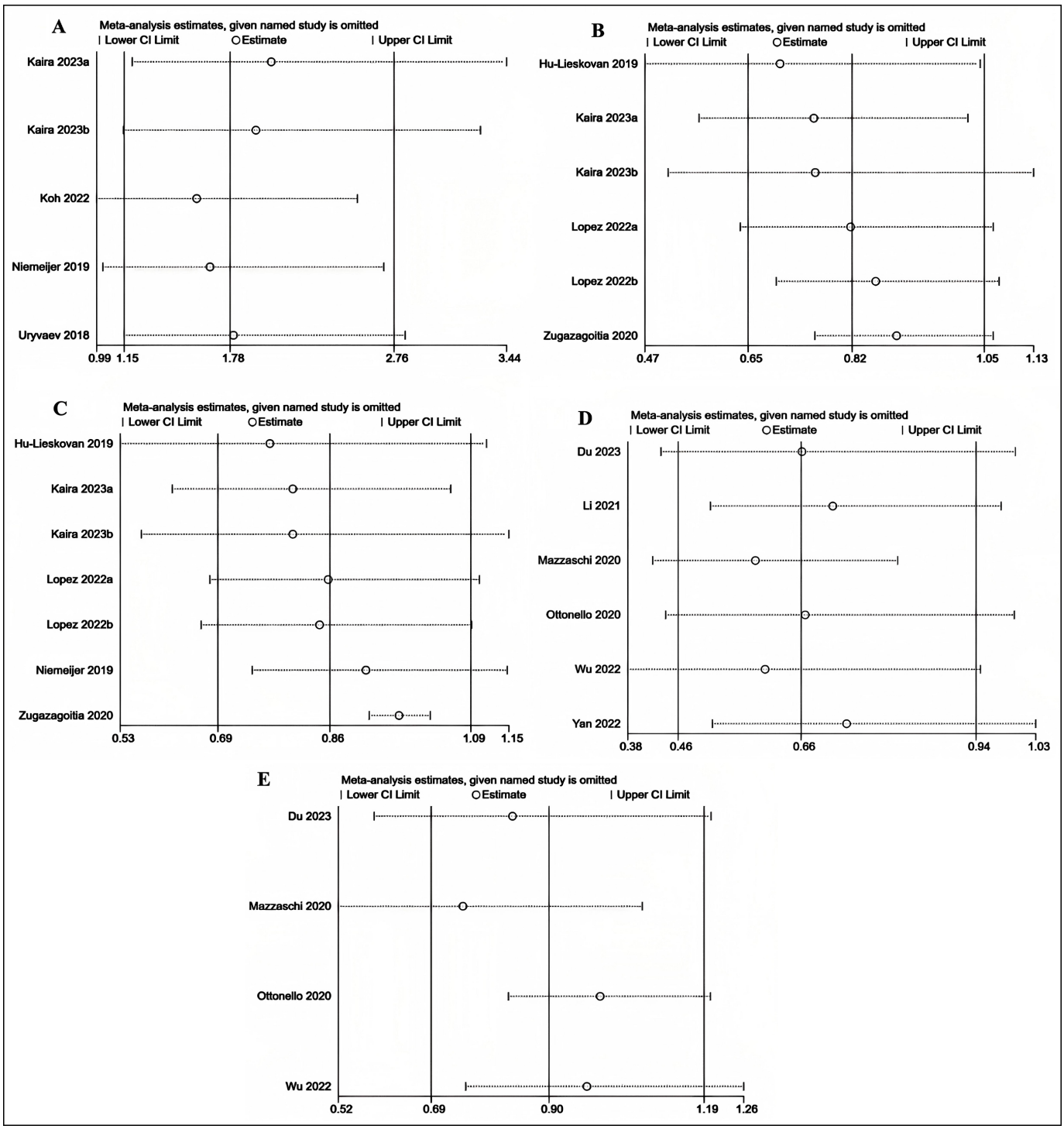

Figure 5: Sensitivity analyses showing the HR and OR. (A) Combined HR of OS from tumour tissue. (B) Combined HR of PFS from tumour tissue. (C) Combined OR of ORR from tumour tissue. (D) Combined HR of OS from peripheral blood. (E) Combined HR of PFS from peripheral blood.

After removing this article, I^2 decreased to 49.0%, the heterogeneity of the p-value became 0.097, HR = 0.70 (95% CI: 0.47-1.04). When studying CD4+ TILs in peripheral blood, the heterogeneity of the combined HR for PFS (I^2 = 68.1%) was from Mazzaschi *et al.*[29] After removing this article, I^2 decreased to 33.6%, the heterogeneity of the p-value became 0.197, HR = 0.59 (95% CI: 0.42-0.81).

DISCUSSION

The advent of immunotherapy has provided new options for the treatment of many cancers, including NSCLC. T cells play a key part in killing tumour cells. Mechanistically, PD-1/PD-L1 inhibitors limit receptor-ligand interactions and antagonise tumour immunity by releasing inhibitory receptors outside T cells. Anti-tumour immunity is enhanced by rescuing the func-

tion of failed T cells through high expression of PD-1.[36,37] Nevertheless, tumour cells use this strategy to evade the anti-tumour immune response, which also constitutes a general condition of immunosuppression. Large numbers of cancer cells upregulate PD-L1 and subsequently, PD-1 attaches to the surface of TILs. This interaction retrains the T-cell response through multiple mechanisms.[38] TILs are a type of special lymphocytes involved in tumour immunity and regulation, which consist of two main components, CD8+ TILs and CD4+ TILs. Peripheral blood or tumour-infiltrating CD8+ T cells have been shown to be related to immune responses to treatment with ICIs.[17] Actually, CD4+ T cells also promote anti-tumour immunity through a variety of mechanisms, including the formation of anti-tumour immunity by recognising the MHC-II-neoantigen complexes,[39,40] providing IL-21 to induce cytolytic chemokines and CD8+ T cells, and promoting CD8+ T cell tropism to the tumour area,[41,42] and exerting immunosuppressive effects through regulatory T cells.[43] CD4+ T cells are the driving force behind the immune cycle of the tumour, as it assists in the initiation and clonal amplification of CD8+ T cells in lymph glands, promote their migration to peripheral blood circulation and tend to the TME.[44] These two types of T cells work together to activate a strong enough anti-cancer immune-killing effect.

According to the findings of this study, the existing evidence suggested that high CD4+ TILs in tumour tissue can predict good ORR for people with NSCLC receiving PD-1/PD-L1 inhibitors. T-cell exhaustion is a marker of tumour specificity and response to immune checkpoint blockade (ICB). Currently, the research on T-cell depletion in cancer patients mainly focuses on CD8 T cell, while CD4 T cell-depletion remains underappreciated. A previous study found a CD4+ TILs population, defined by highly expressed PD-1 and CD39, which contained a high proportion of cytokine-producing cells that, despite the low amount of cytokines produced by these cells, were prone to a depletion state.[45] The next *in vitro* experiments demonstrated that CD4 TIL activation was enhanced, and CD154 expression and cytokine secretion were increased after PD-1 blockade, which promoted dendritic cell maturation, thereby improving tumour-specific CD8 T cell proliferation.[45] The findings suggested that depleted CD4 TILs participate in immune checkpoint blockade responses, which is also defined by hyperexpression PD-1 and CD39 share the same CD8 T cell depletion programme characteristics.[45] By comparing the immune micro-environment changes of patients with and without T cell clonal proliferation before and after treatment, one study suggested that CD4+ and CD8+T cell subtypes expressing PD-1 are the main target cells for anti-PD-1 treatment, and the reaction to PD-1 treatment could be forecast by the level of differentiation and clonal proliferation of the corresponding lineages of CD8+TEX cells and helper CD4+T cells (TH1, TFH). Moreover, PD-1 treatment may further promote the differentiation of such cells.[46] Additionally, after proceeding with multi-omic analyses of changes in the TME, RNA-seq analysis of TIL showed a decrease in primitive CD4+T cells after nivolumab treatment. Meanwhile, the investigators of the study noted that anti-PD-1 therapy reduces lymphocyte aggregation related to the improvement of PD-1+CD4+ and CD8+ T cell OS, induces changes in various cytokine and chemokine signals to activate CD4+ T cells, and can improve its immunogenic activity.[47]

Although tumour tissue-based biomarkers can help determine whether patients are benefiting, many challenges remain in clinical practice. Tumour biopsy is often invasive, and the use of biopsy to obtain patient tissue specimens is greatly confined by tumour availability and patient's condition, especially in patients with advanced cancer. Repeated tissue biopsies may rise the possibility of surgery-related complications and retard cancer therapy. In contrast, peripheral blood collection is minimally invasive, readily available and reproducible. Furthermore, peripheral blood biomarkers could reflect tumour biology and provide details about the host's ever-changing constantly changing immunoresponse to tumours.[48]

Similarly, this study also focused on and explored peripheral blood CD4+ T cells, which revealed that higher CD4+ T cells can predict better PFS for NSCLC patients who receive PD-1/PD-L1 inhibitors. For the past few years, with the application of multicolour fluorescence and mass spectrometry flow cytometry, as well as technological advances in next-generation sequencing to detect various immune cell subpopulations, it has gradually become possible to identify and monitor different circulating immune cells in the peripheral blood. CD4+ T cells can enhance anti-tumour immunisation by promoting the initiation, migration, and survival of CD8+ T cells.[44] In a related study, the researchers used scRNA-seq and scTCR-seq techniques to identify individual peripheral T cell clones and monitor their dynamics during immunotherapy. The results showed that tumour-associated CD4+ T cell clones had more cytotoxic activity compared to CD8+ T cell clones. Based on a large clone of tumour-associated CD4+ T cells, the authors observed a sharp decline in cell abundance after progression and a significant decrease in the proportion of PD-1+ T cells. The above findings help to provide insights into the kinetics of peripheral T cell cloning during PD-1 for NSCLC patients and also reveal the possibility of predictive immunotherapy of CD4+ T cells.[49] Furthermore, a study concluded that the key role of peripheral CD4+ T cell populations rather than CD8 T cells in "real-time" blood monitoring in patients with NSCLC receiving ICIs.[50] The fact that the study was found to show a significant CD4+ T cell profile in the prediction of PD-1/PD-L1 inhibitors monotherapy provided preliminary evidence for the feasibility of combination therapy with immunosuppressive monotherapy in advanced NSCLC patients.[51]

There were some limitations to the study. Exploring sources of heterogeneity through subgroup analysis of a factor was

limited, and more in-depth grouping and stratification warranted further discussion. Suggesting publication bias was based on partial funnel plots and Egger's test. The objective reason may be that research reporting positive findings is relatively easier to get published, and controlling study quality is a prerequisite for meta-analyses. In addition, a single quantitative study of CD4+ TILs was not sufficient to elucidate the complex impact of TME on immunotherapy, and more reliable survival analyses can only be obtained with more rigorous designs, larger sample sizes, and longer follow-up times. Both immunohistochemistry and flow cytometry suffer from technical bias. The above assays and their informatics methods need to be harmonised and the sampling time, sensitivity, and specificity of each test should also be investigated to ensure clinical applicability. The number of included studies was limited. The available results are preliminary. The reliability of the results of this study needs to be reinforced by subsequent clinical research with adequate sample sizes. Cut-off values for standardised CD4+ TILs need to be determined to accurately guide clinical decision-making. An initial cut-off point for CD4+ expression could be determined by the Bayesian approach based on a *priori* information from other trials.[52]

CONCLUSION

This meta-analysis concluded that high CD4+ TILs in tumour tissue were associated with certain favourable prognoses in patients with NSCLC treated with PD-1/PD-L1 inhibitors, regardless of the site of CD4+ TILs. In addition, high CD4+ T cells from peripheral blood were also related to better prognosis. More in-depth studies are necessary to explore the predictive significance of CD4+ TILs in specific subgroups. Finding optimal thresholds and assays for CD4+ TILs through standardised designs is also urgently needed. Moreover, in subsequent studies, researchers should be committed to a single biomarker and rationally combine CD4+ TILs with other eligible biomarkers in order to enhance their predictive power and achieve a relatively promising prognosis for cancer patients receiving immunotherapy.

COMPETING INTEREST:
The authors declared no conflict of interest.

AUTHORS' CONTRIBUTION:
QMZ: Contributed to the design, acquisition, analysis, interpretation of data, and manuscript writing.
YYL, YPW, GXL: Contributed to the result interpretation and discussion writing.
ZGS, MZ: Designed the study.
All authors approved the final version of the manuscript to be published, and agreed to be accountable for all aspects of the work.

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