Systematic Reviews

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Prognostic value of tumour-associated regulatory T-cells as a biomarker in non-small cell lung cancer: a systematic review and meta-analysis

Kapil Khambholja[1†], Manish Gehani[2*†], Rushabh Kothari[3†] and Sachin Marulkar[4†]

Abstract

Background Tumour, nodes, and metastases (TNM) staging has been deficient in prognosticating in patients suffering from non-small cell lung cancer (NSCLC). To supplement TNM staging, this systematic review and meta-analysis aimed to evaluate the prognostic value of the regulatory T cells (Treg).

Methods A keyword search was conducted in MEDLINE and EMBASE for full-text original human studies from any region published in English during the last 12 years. Eligible for inclusion were studies evaluating the prognostic value of the number of Treg cells in NSCLC except case studies, case series, systematic reviews, and meta-analyses. Two reviewers (one reviewer used an automation tool) independently screened the studies and assessed risk-of-bias using the Quality in Prognosis Studies (QUIPS) tool. Meta-analysis was done for studies reporting significant multivariate hazard ratio (HR).

Results Out of 809 retrievals, 24 studies were included in the final review. The low number of Treg cells was found significantly associated with improved overall survival (pooled log OR, 1.646; 95% CI, 1.349, 1.944; p (2-tailed) < .001; SE, 0.1217), improved recurrence-free survival (HR, 1.99; 95% CI, 1.15, 3.46; $p = .01$), improved progression-free survival (pooled log OR, 2.231; 95% CI, 0.424, 4.038; p (2-tailed) .034; SE, 0.4200), and worse disease-free survival (pooled log OR, 0.992; 95% CI, 0.820, 1.163; p (2-tailed) .009; SE, 0.0135), especially when identified by forkhead box P3 (FOXP3), in any stage or non-metastatic NSCLC.

Conclusion A low number of Treg cells indicated better survival, suggesting its potential use as a prognostic biomarker in NSCLC.

Systematic review registration The protocol of this review was prospectively registered on PROSPERO on August 28, 2021, and was assigned the registration number CRD42021270598. The protocol can be accessed from PROSPERO website.

Keyword Meta-analysis, Non-small cell lung cancer, Prognosis, Regulatory T cells, Systematic review

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Background

An estimated 1.8 million deaths and 2.1 million new cases occur annually due to lung cancer around the world [1]. Almost half of the patients die within 1 year of diagnosis and less than 18% survive beyond 5 years [2]. Further, among the patients with non-small cell lung cancer (NSCLC), around two-thirds of patients fail to survive beyond 2 years [3, 4].

The dismal prognosis is exacerbated by a lack of methods for early diagnosis and prognostication and limited access to opportune standard treatment. Historically, the clinical fraternity has relied upon the tumour, nodes, and metastases (TNM) staging as the gold standard prognostic tool for lung cancer [5], although, several researchers have raised concerns about various editions of the TNM classification [6–9]. Recently, several studies have reported a profound prognostic impact of tumour-infiltrating lymphocytes (TILs) in malignant tumours [10–17] including lung cancer [18]. Further, immune scoring based on TILs or their ratios for differentiating prognosis within each tumour, node, and metastasis has been used to enhance the prognostic value of TNM staging [19–22].

TILs present in the immune infiltrate called the tumour microenvironment (TME) include effector T cells, which can be T helper cells (1, 2, and 17), regulatory T (Treg) cells, T follicular helper cells, and cytotoxic T cells [23–25]. These cells may be localized in the tumour parenchyma, invasive margin, or adjacent tumour stroma, where they interact with tumour cells in three phases of immuno-editing [26]. During the escape phase, tumour cells induce the production of cytokines and growth factors and facilitate the recruitment of immunosuppressive cells, thereby causing immune suppression [27–29].

Tregs are a highly immune-suppressive subset of clusters of differentiation (CD) 4^+ T cells [30–32], which play an important role in the preservation of self-tolerance and modulation of the overall immune responses against tumour cells through numerous cellular and humoral mechanisms [33, 34]. Studies have shown that the composition of Tregs is altered in the TME, where the effector Treg numbers are increased in NSCLC patients as compared to healthy individuals [35, 36]. Transforming growth factor (TGF) -β1 and interleukin (IL) -2 pro-inflammatory cytokines which are present at a high level in tumour tissues of NSCLC patients promote the differentiation of naïve T-cells into Tregs [35]. Moreover, due to self-antigens released in the TME by dying cancer cells, nTregs are converted into effector Tregs by expressing a higher level of activation biomarkers [35]. In a study, Erfani et al. reported that the NSCLC patients had almost twice Treg cells than the healthy controls [37]. Further, they reported that the metastatic stages had almost two times more Treg cells than the non-metastatic stages [37]. This makes Tregs an ideal therapeutic target and candidate for prognostication of lung cancer, especially NSCLC, which comprises about 85% of all lung cancer cases [38]. While the therapeutic targeting of Treg by pathways like the blockade of immune checkpoint molecules has been studied by many authors [33], the prognostic value of measurement and localization of Treg cells has not been fully evaluated in all study populations, with various prognostic factor variables, causing individual studies to report piecemeal and mixed results.

The objective of the current systematic review and meta-analysis was to evaluate whether the "number of Treg cells" has any prognostic value in predicting the survival of NSCLC patients in various study populations, considering varied prognostic factor variables and survival outcomes.

Methods

The study conducted adheres to and is in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki 1975, as revised in 2000) for experiments involving humans. The systematic review did not deal with any patient-level data or disclose any identifiable patient data; hence Institutional Review Board Approval or Informed Consent of the patients was not sought. The protocol of this review was prospectively registered on PROSPERO on August 28, 2021, and was assigned the registration number CRD42021270598. The protocol can be accessed from the PROSPERO website. This manuscript is written in compliance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guideline. The PRISMA 2020 checklist for this submission can be found in additional files (Additional file 4).

Design and setting

The design of the current study was a systematic review and meta-analysis. An online electronic database search was conducted from July 19 to 25, 2022. An update of the systematic review was conducted from July 20 to August 5, 2024, to capture the newly published studies. Retrieved studies underwent screening, quality assessment, and extraction of the required information. The systematic review process was facilitated by an automation tool "MAIA", an artificial intelligence-based proprietary platform developed in-house by Genpro Research Pvt Ltd. MAIA tool was used to search on PubMed and retrieve the abstract and publicly available articles through PubMed Central or respective journal portals. MAIA was further used to support screening and data extraction where MAIA's Natural Language Processing highlighter helped to locate the relevant information quickly, thereby helping in the final decision of relevant information

capture. All these steps were machine-augmented, giving the final decision to the reviewer. The tool was not equipped to search EMBASE till the time this manuscript was written.

Search strategy

To fulfil Methodological Expectations of Cochrane Intervention Reviews (MECIR) Standards, the review was conducted in two databases [39]. PubMed was used to conduct the search in the MEDLINE database, parallelly by a manual reviewer and another reviewer using the automation tool "MAIA". Retrievals were sought for the relevant published prospective as well as retrospective studies describing the prognostic value of presence, degree, measurement, or localization of Tregs in tumour tissue or body fluids of patients with NSCLC, through histological or cytological procedures like immuno-histochemistry, flow cytometry, or quantitative real-time polymerase chain reaction, assessing either Treg alone or in combination with at least one biomarker out of $CD4^+$, $CD25^+$, $CD127^-$, $Helios^+$, and/or forkhead box P3 $(FOXP3)^+$ [33]. EMBASE was searched and reviewed manually by the two reviewers.

Free full-text studies presenting original research in humans, published in English in any region were included in the review. Studies published during the last 12 years were included, so as to ensure that the recent advances in standard of care for laboratory testing were adequately represented in the review. Protocols or design papers, education literature, and meeting reports were excluded. Letters to the editor and brief communications were included only if they described original research with its results. Designs such as case studies, case series, systematic reviews, or meta-analyses were excluded. As pre-specified in the protocol submitted to PROSPERO prior to the conduct of the review, studies that failed in the quality assessment were excluded from the review. Cross-references from review articles were reviewed to ensure that no eligible article was missed. The search was not rerun before the final analysis. No unpublished study was sought so as to allow comparison of results between the manual review and the automation tool "MAIA", which was equipped to evaluate only the published studies. The last search was done on July 20, 2024.

Using predefined eligibility criteria based on the "Population, Prognostic Factor, Outcome" (PFO) approach (Additional file 1), two reviewers screened the unblinded titles, abstracts, and full text of the studies independently in a standardized manner. One reviewer performed screening manually using Microsoft Excel (v2403), while the second reviewer used "MAIA" automation tool. The disagreements were resolved by consultation with other co-authors. Before the resolution of disagreements, the agreement analysis showed the kappa as 0.819, while after the resolution of disagreements, the kappa was 1.000 (Additional file 5).

The Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) [40] Flow Diagram was created based on the retrieved literature, screened records, excluded studies, and included studies (Fig. 1). For the quality assessment of the studies after screening, Cochrane's Quality in Prognosis Studies (QUIPS) tool was used. Only those studies were included for extraction of data for the final narrative synthesis and quantitative analysis that showed a low risk of bias for "study participation", "prognostic factor measurement", and "outcome measurement" domains, while low to moderate risk of bias for "study attrition", "study confounding", and "statistical analysis and reporting" domains. Data extraction was done by the first reviewer and verified by a second reviewer. The automation tool "MAIA" was used to facilitate the extraction of data. The detailed search strategy including keywords used, retrieval, eligibility criteria, risk of bias assessment, and extraction are provided in Additional file 1.

Statistical analysis

The primary outcome measured by this review was how the number of Treg cells affected the survival of the patients with NSCLC in terms of overall survival (OS), progression-free survival (PFS), disease-free survival (DFS), or recurrence-free survival (RFS), with point estimates as risk and ratios such as relative risk (RR), odds ratio (OR), or hazards ratio (HR). OS was defined as the time between the first diagnosis of NSCLC and the date of death regardless of the cause. PFS, DFS, and RFS were defined as the time between the date of diagnosis and the date of progression, recurrent symptoms, and first relapse respectively. The secondary outcome of the study was to quantify recurrence and metastasis.

The data were pooled for quantitative synthesis from the individual studies through meta-analysis using Statistical Package for the Social Sciences (SPSS) (v29.0.2.0). As per the Cochrane Consumers and Communication Group review for meta-analysis, a minimum of two studies with the same type of survival outcome were required to subject them to quantitative synthesis. In case enough studies were not available for certain survival outcomes, findings from the individual studies were summarized in the narrative.

The survival analysis was reflected by the individual studies as HR, 95% confidence interval (CI), and p-value. Studies with a significant p-value (< 0.05) for multivariate HR were considered for meta-analysis to avoid bias due to confounding by co-factors. Studies

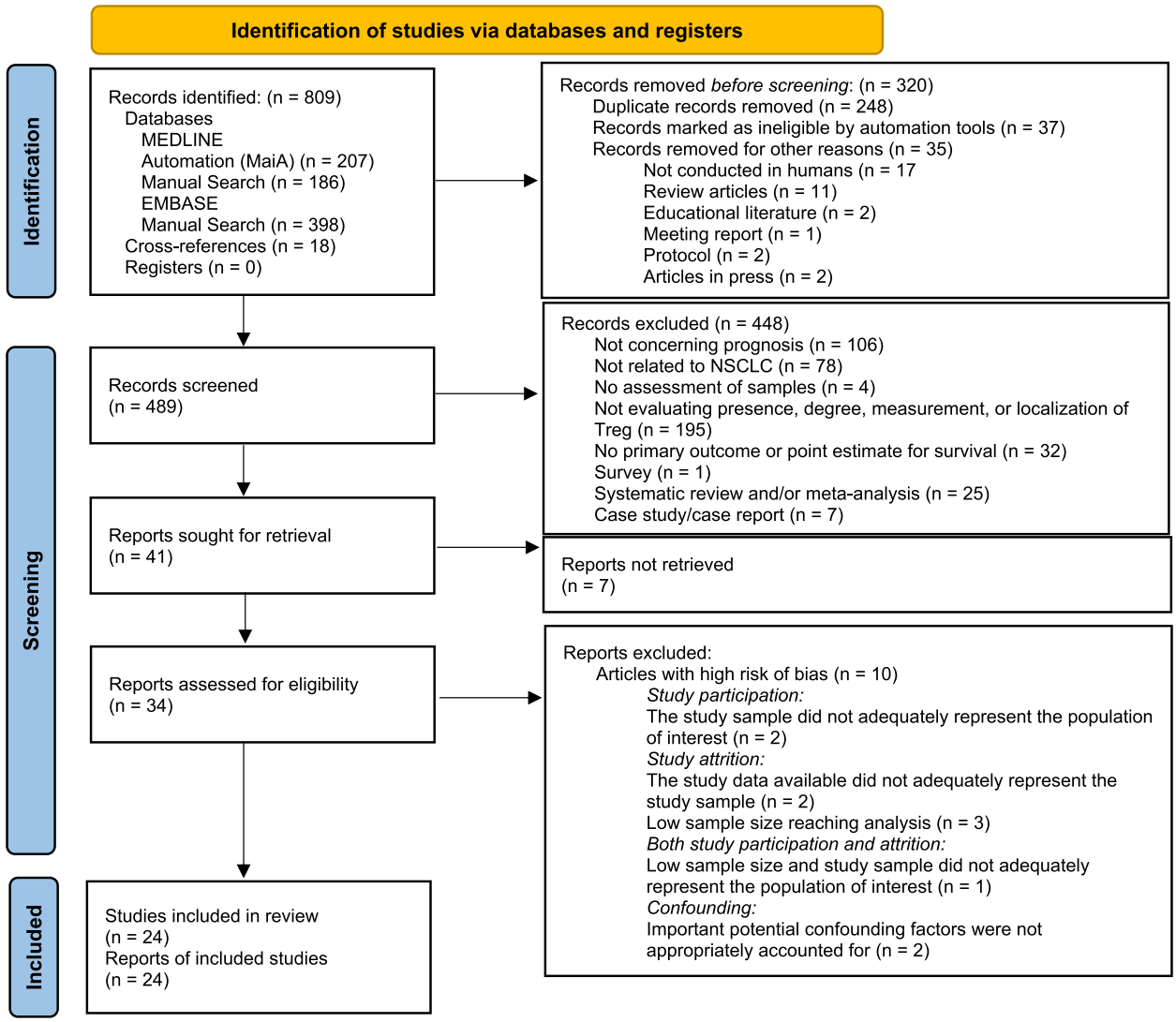

Fig. 1 PRISMA 2020 diagram: flow of the studies through the review process

having a low number of Tregs as the reference category for HR were eventually included in the meta-analysis.

For pooling HRs, a meta-analysis for binary outcomes with pre-calculated effect sizes was run in SPSS. Confidence intervals were converted to variance. Since the variables related to the study population, prognostic factor, outcome, and study design were not completely similar among the included studies, the random effect model was applied. Inverse-variance method was used to determine the weight of the studies. DerSimonian-Laird was used as the estimation method. Standard error adjustment was done using the Knapp-Hartung adjustment method. The low number of Treg became the control category for the pooled OR. A sensitivity analysis for the standard method was also run but eventually, the significant results obtained from the Knapp-Hartung adjustment method were reported in the results. The summary effect estimate (log OR) and its confidence interval were determined by generating forest plots using the HRs and associated variance for survival outcomes. The I^2 statistic was calculated for determining heterogeneity where an I^2 value lower than 30% was considered indicative of homogeneity. The symmetry of funnel plots generated using effect size and variance was used to assess publication bias visually. No sub-group analysis was performed. The data generated during screening, risk-of-bias assessment, and extraction process was uploaded in the online Harvard Dataverse Repository.

Results

Included studies: flow through the review and characteristics

Out of 809 retrievals obtained through automation, manual search, and cross-references, 320 studies were filtered out, 448 studies were removed by screening, and seven studies were not available in full text.

Further, ten studies failed the risk of bias assessment using the QUIPS tool of Cochrane and were excluded from the final quantitative analysis. Out of these studies, most (5/9) failed to fulfil the criteria for the *study attrition domain*: while four studies had low sample size reaching the analysis stage, in another study, the available data did not adequately represent the study sample. Further, two studies could not fulfil the criteria for the *study participation domain* as their study sample did not adequately represent the population of interest. Another two studies could not fulfil the criteria for the *study confounding domain* since the important potential confounding factors were not appropriately accounted for, whereas one study failed to fulfil the criteria for both *study participation and study attrition domains* due to low sample size and lack of representative study sample. All the studies fulfilled the criteria for the domains of *prognostic factor measurement, outcome measurement, and statistical analysis and reporting*. The granular details of the risk of bias assessment are shared as Additional file 6. Eventually, 24 studies (18 from MEDLINE manual search, three from EMBASE manual search, and three from cross-references) were included in the review (Fig. 1) [41–64].

Most studies had the study population as non-metastatic NSCLC ($n=7$) or any NSCLC ($n=5$). Most of the studies included all sub-types of NSCLC ($n=20$). Six studies included patients who underwent surgery without any neo-adjuvant therapy, two studies included patients who did not receive any adjuvant therapy, one study enrolled patients treated with stereotactic ablative radiation therapy, while one study reported on patients who underwent first-line treatment with anti-PD1 with or without chemotherapy. Most studies performed tests on Formalin-fixed paraffin-embedded (FFPE) sections or preserved tissue-microarray blocks, either alone or with other types of samples ($n=15$), commonly using the immuno-histochemistry method ($n=14$). FOXP3 ($n=9$) was the commonest biomarker used. Most studies reported OS ($n=18$) either as the only survival outcome or along with other outcomes. Twenty studies (83.3%) were designed as an analytical cross-sectional study with or without a comparison group. Out of the nine studies with a comparison group (Cross-sectional: 6, Cohort: 2, Controlled before-after: 1), six used healthy volunteers (three used age-matched healthy volunteers, one used both age- and sex-matched healthy volunteers, and two studies used unmatched healthy volunteers), while one used cancer-free cardiology patients matched for age, demographic characteristics, and comorbidities. No repeated measures of laboratory parameters were done in cross-sectional studies, but a longitudinal follow-up component was embedded only for survival events such as death, progression, recurrent symptoms, or first relapse. Fourteen studies were retrospective in nature, while eight were prospective, and two studies were both prospective and retrospective. Report characteristics and study characteristics of the included studies are detailed in Table 1. The study-wise details of report characteristics, population under study, prognostic factor, outcome, and study design are provided in Additional file 7.

Quantitative synthesis of the study results

Out of the 24 included studies, only 13 studies, reported significant ($p \leq 0.05$) multivariate analysis. Among these studies, 11 studies reported 14 OS values, of which, eventually, seven OS values from seven studies were included in the meta-analysis (Fig. 2a). The population for these studies were non-metastatic NSCLC ($n=2$), any stage NSCLC ($n=2$), naïve NSCLC ($n=1$), pulmonary recurrence-based oligometastatic NSCLC treated with stereotactic ablative radiation therapy (SABR) ($n=1$), and NSCLC with Karnofsky Performance Status (KPS) > 80% ($n=1$). Four studies measured Treg in FFPE sections by immuno-histochemistry, while three studies measured the same in peripheral blood using flow cytometry. Four studies measured FOXP3 as the biomarker for Treg and three studies measured multiple biomarkers. All seven studies ran the Cox proportional hazards model for survival analysis. Six of them were analytical cross-sectional in design, while one was a cohort study. All seven studies had no repeated measurements of laboratory parameters and had a longitudinal follow-up component only for survival outcomes. Three of these studies were prospective and four studies were retrospective.

Among the 13 studies reporting significant multivariate analysis, eventually, two studies reporting two values for DFS were included in the meta-analysis (Fig. 2b) and three studies reporting three values of PFS were included in the meta-analysis (Fig. 2c). Only one study reported RFS among the ten studies reporting significant multivariate analysis. Since a minimum of two studies were required for performing a meta-analysis, quantitative synthesis could not be done for RFS.

Out of the final 24 studies included in the review, except one study which evaluated the $FOXP3^{+}$ category and the FOXP3- category in non-metastatic NSCLC, all the remaining 23 studies assessed a high and low Tregs; however, the low Treg was defined differently in different studies as described in Table 2.

Table 1 Characteristics of the included studies

Report characteristics	*n*	Study characteristics	*n*	Study characteristics	*n*
Year of publication (N = 24)		***Population under study (N = 24)***		*Biomarkers used for Treg (N = 24)*	
2014	1	Any NSCLC	5	FOXP3 +	9
2015	1	Any stage NSCLC	1	CD4 + CD25 + / + + CD127 − /dim	5
2016	5	Chemotherapy-naïve any stage NSCLC	1	CD4 + FOXP3 +	3
2017	4	Newly diagnosed any stage NSCLC	1	CD4 +	1
2018	1	Stage 1 NSCLC	1	Helios + FOXP3 +	1
2019	4	Non-metastatic NSCLC	7	CD3 + FOXP3 +	1
2020	3	Chemotherapy-naïve non-metastatic NSCLC	1	Treg + TLS-DC + CD8	1
2021	3	Metastatic NSCLC	2	CD8 + FOXP3 +	1
2022	1	Pulmonary recurrence-based oligometastatic NSCLC treated with SABR	1	CD3 + CD45RO + FOXP3 +	1
2024	1	First diagnosis and relapsed NSCLC	1	No biomarker	1
Language (N = 24)		NSCLC with KPS > 80%	1	***Outcomes (N = 24)***	
English	24	Resected NSCLC without neo-adjuvant chemo-radiotherapy	1	Overall survival	8
Region (N = 24)		Solitary lesion in localized NSCLC	1	Disease-free survival	3
China	9	***Prognostic factor (N = 24)***		Progression-free survival	3
Japan	3	*Samples for Treg assessment*		Recurrence-free survival	1
Brazil	2	FFPE sections or preserved tissue-microarray blocks	12	Both overall survival and disease-free survival	3
Germany	2	Peripheral blood	7	Both overall survival and progression-free survival	3
France	2	Both FFPE and peripheral blood	2	Both overall survival and recurrence-free survival	3
Greece	1	Fresh surgical specimens	1	***Study design (N = 24)***	
Australia	1	FFPE and fresh tumour biopsies	1	Analytical cross-sectional design	14
Spain	1	FFPE, fresh tumour biopsies, non-tumoral distant lung specimens, lymph nodes specimens, and blood	1	Analytical cross-sectional design with comparison group	6
United States of America	1	*Tests conducted on samples*		Longitudinal	1
Poland	1	Immuno-histochemistry	9	Controlled before after study	1
Italy	1	Flow cytometry	6	Cohort study	2
Species (N = 24)		Flow cytometry and immuno-histochemistry	4		
Humans	24	Multiplex immuno-fluorescence staining	2		
Article type (N = 24)		Immuno-histochemistry with FOXP3 staining	1		
Original research article	23	Flow cytometry and antibody staining	1		
Letter to the editor	1	Immuno-histochemistry and immuno-fluorescence staining	1		

CD Clusters of differentiation, *FFPE* Formalin-fixed paraffin-embedded, *FOXP3* Forkhead box P3, *KPS* Karnofsky Performance Status, *NSCLC* Non-small cell lung cancer, *SABR* Stereotactic ablative radiation therapy, *TLS-DC* Tertiary lymphoid structures-dendritic cell, *Treg* Regulatory T cell

Summary estimates from the meta-analysis

The seven studies analysed for OS had a Q value less than the degree of freedom (K-1); hence, I^2 was zero and the included studies had low heterogeneity. The summary log OR was 1.646; 95% CI, 1.349, 1.944; p (2-tailed) < 0.001; standard error (SE), 0.1217 (Fig. 3a). The two studies pooled for DFS had low heterogeneity ($I^2 = 0$), and the summary log OR was 0.992; 95% CI, 0.820, 1.163; p (2-tailed) 0.009; SE, 0.0135 (Fig. 3b). The three studies pooled for PFS had low heterogeneity ($I^2 = 0$), and the summary log OR as 2.231; 95% CI, 0.424, 4.038; p (2-tailed) = 0.034; SE, 0.4200 (Fig. 3c). The one study which evaluated RFS reported multivariate HR as 1.99; 95% CI, 1.15, 3.46; $p = 0.01$. The funnel plots for OS, DFS, and PFS did not show any publication bias as depicted in Additional file 3.

The studies included in the review also reported other results such as quantification, localization, recurrence, metastasis, and correlation of programmed cell death protein 1 (PD-L1) with the level of Tregs. These results are provided in an Additional file 2.

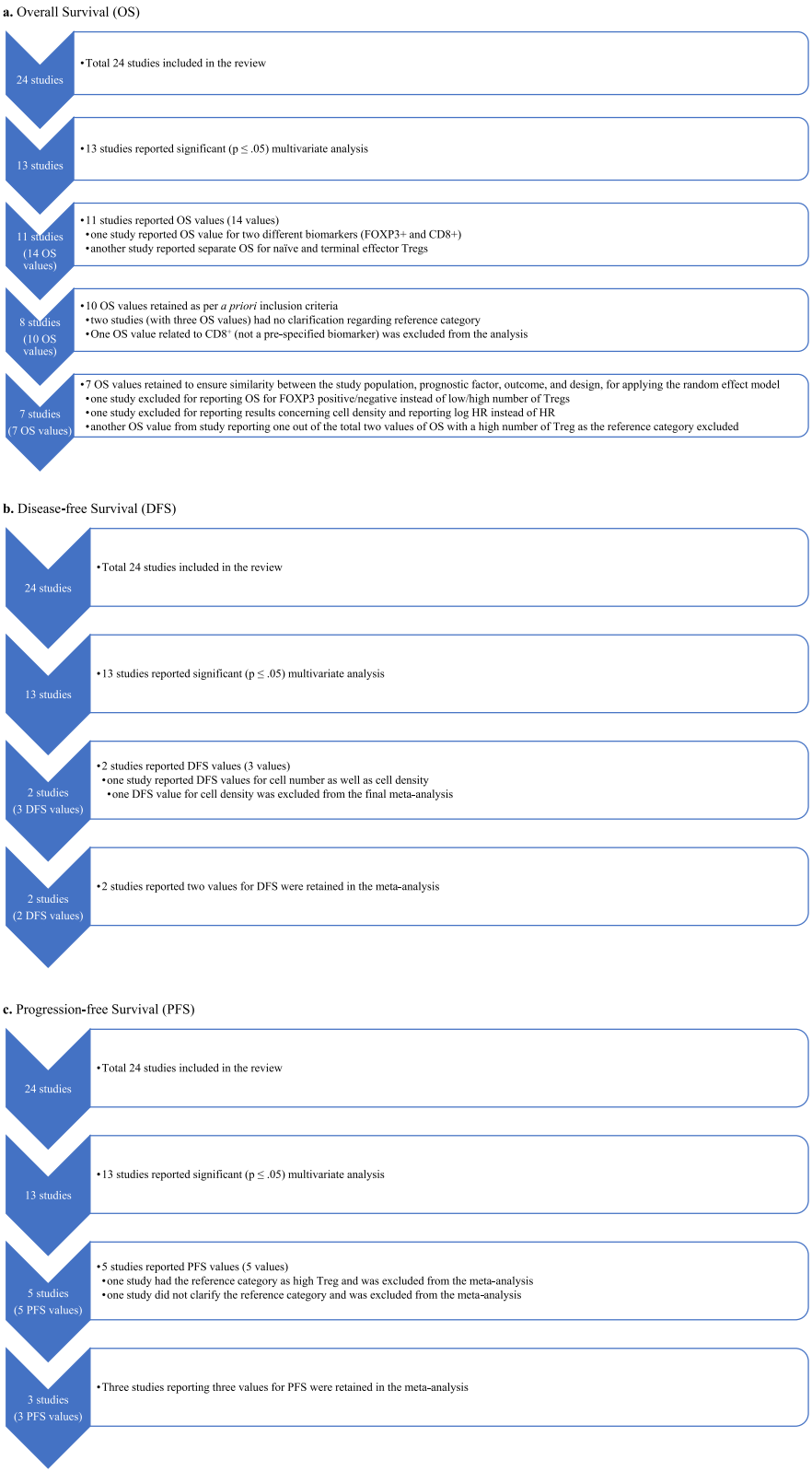

Fig. 2 Selection of studies for quantitative synthesis. **a** Overall Survival (OS). **b** Disease-free Survival (DFS). **c** Progression-free Survival (PFS)

Table 2 Definitions of low Treg used in different studies for multivariate analysis

Study population	Definition of low Treg used in different studies ($n=23$)
Only stage 1 NSCLC patients	• Low cell counts of less than 45 per high power field ($n=1$)
Only stage 1 and 2 NSCLC patients	• Not specified (although text mentions high FoxP3, but no cut-off is provided) ($n=1$)
Non-metastatic NSCLC patients	• Less than 10% of the total lymphocyte count ($n=1$) • Low area under the curve than a cut-off ($n=1$) • Lower than the median ($n=1$) • Below the optimal cutoff point according to the built-in risk scoring formula in X-tile ($n=1$) • Not specified (although text mentions low FoxP3, but no cut-off is provided) ($n=1$) • Not specified (although the text mentions the comparative frequency of Tregs in patients and healthy controls) ($n=1$)
Only metastatic NSCLC patients	• Lower than the median ($n=1$) • Not specified ($n=1$)
NSCLC patients irrespective of the stage	• Lower than the median ($n=2$) • Lower than mean ($n=2$) • Lower than a cut-off concerning percent of CD4 count ($n=2$) • Less than 95% of controls ($n=1$) • Low concentration in tumour tissue than a pre-specified level ($n=1$) • High score (score 2–3) and low score (score 0–1), calculated as per the proportion of positively stained cells out of the total nucleated cells ($n=1$) • Below 25 in number ($n=1$) • Unspecified ($n=2$)
Patients with pulmonary recurrence-based oligometastatic NSCLC treated with SABR	• Lower than the median ($n=1$)

Discussion

The results of the review indicate that the number of Treg cells was significantly associated with OS ($p<0.001$), DFS ($p=0.009$), and PFS ($p=0.034$) in NSCLC. The pooled log OR of more than one indicates that the reference category as a low number of Treg was associated with improved OS. On the contrary, DFS was better with the high number of Tregs, although the analysis pooled only two studies and both used different biomarkers and study populations. The low number of Tregs also showed higher PFS. Based on one study that reported significant HR for RFS, a low number of Treg had significantly better RFS ($p=0.01$). The log OR generated by the forest plot for OS was significant for the study population "any stage NSCLC" and FOXP3 as the biomarker, while the one generated for DFS was significant for the study population "any stage NSCLC" and "non-metastatic NSCLC" and biomarker as FOXP3 and $CD4^{+}FOXP3$. The study reporting significant HR for RFS also evaluated FOXP3 in any stage NSCLC. Overall, the meta-analysis reflected that a low number of Treg cells indicated better survival, especially in any stage NSCLC or non-metastatic NSCLC, while using FOXP3 as the biomarker.

Most of the past studies reported findings similar to our study, although few studies contradicted our conclusion. Peng et al. concluded that a low number of Tregs had significantly poor DFS [42]. Kotsakis et al. found that a high number of terminal effector Tregs was associated with significantly better OS and PFS [51]. On univariate Cox regression, Muto et al. reported significantly better OS [54], while Ameratunga et al. reported significantly better DFS in patients with a high number of Treg cells [45]. None of the previous studies could highlight the role of Tregs in different study populations, considering different prognostic factor variables, outcomes, and study designs. None of the published reviews on this topic was designed as a meta-analysis and/or a systematic review[34, 65]. On the contrary, our review was designed as a systematic review and meta-analysis and was comprehensive with respect to varied study populations, prognostic factor variables, outcomes, and several other aspects.

The current study had certain limitations. Searching only two portals might have introduced bias, although the unavailability of filters appropriate for clinical studies in other search portals like Scopus and Google Scholar discouraged the authors from including them in the search strategy. Since MEDLINE and EMBASE are the biggest databases for clinical studies, the authors chose to conduct the review on the PubMed portal and the EMBASE portal.

Variations in study populations, prognostic factor variables (such as types of Tregs, biomarkers, samples, and procedures of measurement), outcomes, study designs, analytical tests, reference categories, quantification, and localization of Tregs, among the included studies may have introduced imprecision in results and may be a source of bias. Additionally, due to a lack of data in the included studies for variables such as race and ethnicity, the population heterogeneity could not

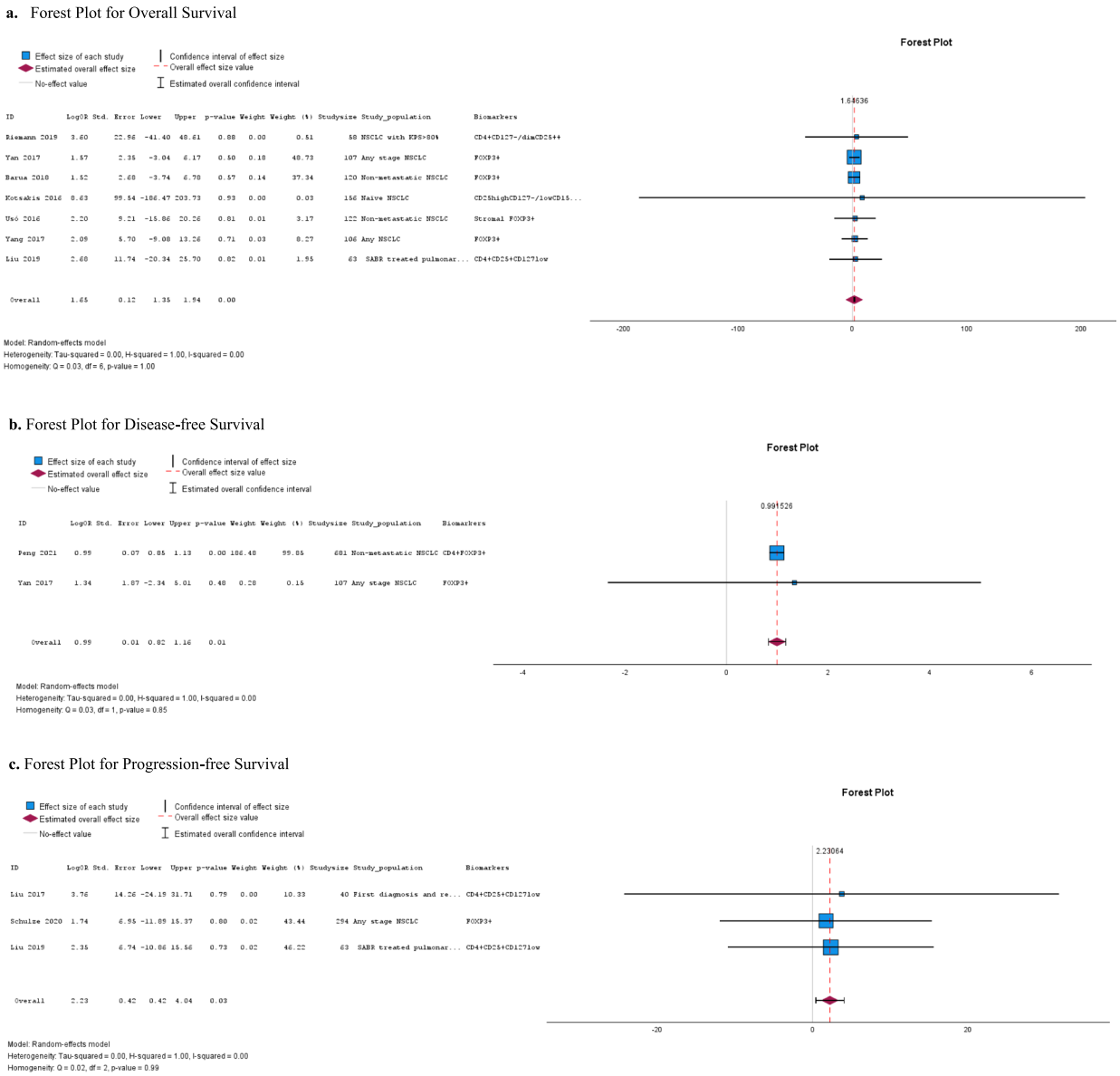

Fig. 3 Forest plots generated by meta-analysis. **a** Forest Plot for Overall Survival. **b** Forest Plot for Disease-free Survival. **c** Forest Plot for Progression-free Survival

be accounted for in the studies gathered from different regions.

Several factors influence the function of Tregs in the TME by acting on the development, maturation, and differentiation of Tregs, or by providing favourable conditions for the functioning of Tregs [65–74]. Moreover, multiple mechanisms of action of Tregs pose difficulty in explaining the exact mechanism contributing to the results of the individual studies [33, 34]. Since the current review did not assess any of these factors influencing the function of Tregs or the underlying mechanisms, they could have potentially confounded the results. Further, due to the low number of studies included in the final meta-analysis, sub-group analysis could not be conducted for many variables such as study population and biomarkers. Nevertheless, the present review applied meta-analysis to synthesize the best available evidence for the use of the number of Treg cells as a prognostic indicator in NSCLC.

Conclusions

Based on the results of this meta-analysis, authors recommend using the "number of Treg cells" as a prognostic biomarker, especially in any stage NSCLC or non-metastatic NSCLC, while using FOXP3 expression as the specific marker, although further experimental studies designed for various populations, biomarkers, and outcomes are needed to confirm these findings. By highlighting the significance of Treg cells in the prognosis of NSCLC, this study can pave the way for developing better prognostic tests, supplementing the TNM staging-based clinical-decision making, and formulating novel therapeutic approaches in cancer treatment.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13643-024-02642-w.

Supplementary Material 1: Additional Note 1. Detailed Search Strategy, Risk of Bias Assessment, and Data Extraction.

Supplementary Material 2: Additional Note 2. Other Results Reported by the Studies Included in the Review.

Supplementary Material 3: Additional Figure 1. Funnel Plots. Figure 1a. Funnel Plot for Overall Survival. Figure 1b. Funnel Plot for Disease-free Survival. Figure 1c. Funnel Plot for Progression-free Survival.

Supplementary Material 4: Additional Table 1. PRISMA 2020 checklist.

Supplementary Material 5: Additional Table 2. Agreement Analysis Sheet.

Supplementary Material 6: Additional Table 3. QUIPS Assessment Results.

Supplementary Material 7: Additional Table 4. Study-wise PFO Table.

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Authors' contributions

KK contributed to concept and design, investigation, project administration, and critical revision of the manuscript. MG contributed to acquisition of data, investigation, and methodology. MG analysed the data and wrote the original draft of the manuscript. SM contributed to concept and design, critical revision of the manuscript, and supervised the review. RK contributed to interpretation of data, validation of findings of the review, and critical revision of the manuscript. All authors read and approved the final manuscript.

Authors' information

KK is Ph.D., MG is M.B.B.S, Ph.D., RK is D.M. Oncology, and SM is M.D.

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Availability of data and materials

The datasets generated and/or analysed during the current study during screening, risk-of-bias assessment, and extraction process are available in the online Harvard Dataverse Repository, https://doi.org/10.7910/DVN/JQOI9V [75].

Declarations

Ethics approval and consent to participate

The study conducted adheres to and is in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki 1975, as revised in 2000) for experiments involving humans. The systematic review did not deal with any patient-level data or disclose any identifiable patient data; hence Institutional Review Board Approval or Informed Consent of the patients to participate was not sought.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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