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Regulatory T cells are an important prognostic factor in breast cancer: a systematic review and meta-analysis

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The clinical relevance of regulatory T cell (Treg) infiltration in breast cancer (BC) remains controversial, and no recent meta-analysis has been published on this subject. Our aim was to identify the precise relationship between Tregs and the prognosis and clinic-pathological features of BC. Eligible articles were identified with a MEDLINE database search over a period up to March 2015. Our meta-analysis was performed using STATA software 11.0 and Review Manager 5.3. The correlations between Treg infiltration and clinico-pathological features and BC prognosis were analyzed. Subgroup and sensitivity analyses, as well as meta-regression, were conducted. Eighteen published studies (including 8,562 patients) were eligible. Overall survival (OS) and disease-, recurrence-, and progression-free survival (DFS/RFS/PFS) were correlated with Treg infiltration (OR=2.03 (95% CI, 1.40–2.95; P=0.000) and 1.48 (95% CI, 1.00–2.19; P=0.050), respectively), including 3-, 5-, and 10-year mortality rates. In addition, low Treg infiltration was present in estrogen receptor (ER)-positive tumors (P=0.000), progesterone receptor (PR)-positive tumors (P=0.003), Her2-negative tumors (P=0.000) and histological grade I/II tumors (P=0.001). No publication bias was observed with the exception of OS. Subgroup analysis suggested that the mortality rate of the high Treg infiltration subgroup was increased compared with the low Treg infiltration subgroup among ER-positive patients. Treg infiltration indicated a poorer prognosis for BC and is related to ER, PR, and Her2 status and histological grade. Thus, Treg infiltration could help predict outcomes and guide clinical therapy.

Key words: breast cancer, regulatory T cells, prognosis, estrogen receptor status, meta-analysis

Breast cancer (BC) is the most common cancer in women, with over 231,840 new cases and 40,290 deaths estimated to have occurred thus far in 2015 [1]. In recent decades, despite earlier detection and more effective treatment approaches, which have improved the survival rate, current data indicate that advanced malignant disease remains far from being therapeutically controllable. In addition, traditional therapy, including surgery, radiation, and chemotherapy, often cause various side effects and fail to remove the tumor completely [2]. Immunotherapy is a promising approach to treating patients with invasive and metastatic BC [3, 4]. However, the immunosuppressive microenvironments induced by regulatory T cells (Treg) in BC present major barriers to successful antitumor immunotherapy [3, 4].

Tregs, which typically express CD25, are naturally present in the immune system, accounting for 5 to 10% of $CD4^+$ T cells. The forkhead/winged helix transcription factor gene *FOXP3* is expressed by Tregs and has been considered a key regulatory gene for the development and function of Tregs [5]. Tregs are important in the control of immune responses by suppressing T cell proliferation and cytokine production and serve as regulatory factors in the tumor microenvironment [6, 7]. Tregs exhibit prognostic value in patients with gastrointestinal cancers and hepatocellular carcinoma [8, 9] and are associated with an unfavorable prognosis. This value is based on their capacity to inhibit antitumor immunity. However, high tumor infiltration by Tregs can improve survival in some tumors [10-12]. The value of Tregs in BC remains controversial. Some studies have not observed any correlation between Treg infiltration and prognosis [13, 14]. In contrast, other studies have found associations with a poor response to chemotherapy and poor clinical outcomes in BC patients [15, 16]. In addition, high Treg numbers are associated with

a higher tumor grade [17], estrogen receptor (ER) negativity [18], Her2 positivity [19] and lymphatic metastasis [20]. To the best of our knowledge, no published meta-analysis has examined the prognostic value of Treg infiltration exclusively in BC. Therefore, we performed this meta-analysis to explore the clinical utility of Tregs in BC based on the conflicting results from previous studies.

Materials and methods

Literature search. Relevant articles were identified by two reviewers (Y. W. and J. T. S.) by electronically searching the PubMed, EMBASE, and Chinese CNKI databases using the following keywords: "Foxp3, CD25, regulatory T cells or Treg," "breast cancer or breast tumor" and "prognosis, prediction, survival or outcome". The period of the search was from inception to March 30, 2015. Additionally, possible overlooked papers were searched from the reference lists of systematic reviews and selected papers.

Selection criteria. Studies included in this meta-analysis evaluated the association between Treg infiltration and BC. The criteria for inclusion were as follows: (a) patients clearly diagnosed with BC; (b) report of Tregs in tumor surgical specimens evaluated via immunohistochemistry (IHC); (c) articles evaluating the relationships between Treg infiltration and clinico-pathological features and prognostic factors of BC, such as overall survival (OS), breast cancer-specific survival (BCSS), progression-free survival (PFS), recurrence-free survival (RFS) and/or disease-free survival (DFS), and contained survival curves; (d) articles containing sufficient published data to estimate a hazard ratio (HR) and a 95% confidence interval (95% CI); and (e) articles published in English. The exclusion criteria included (a) letters, reviews, case reports, conference abstracts, editorials, expert opinions, duplicated studies, articles published in books, and papers published in non-English languages; and (b) non-primary cancer, such as metastatic cancer.

Data extraction. Data were extracted by study authors Wang and Zheng, and discrepancies were resolved by discussion with a third reviewer (X. Q.) until a consensus was reached. The following information was extracted from each eligible trial: the first author's name, publication journal and year, sample size (total and for each arm), country, type of patients, quality score, primary end point, Treg staining method, Treg marker, Treg staining location, duration of follow-up, cutoff point, data collection methods, survival analysis and clinico-pathological parameters.

Study quality and risk of bias assessment. Study quality was assessed with an established form, which was first developed by Hayes [21] and used by McShane [22]. The evaluation contents are provided in Supplementary Table 1 and were scored on a scale from 0 to 8.

Definition of prognostic outcomes and subgroup analyses. OS was defined as the time from surgery until death. BCSS was defined as the time from the initial diagnosis of BC to death attributed to BC. RFS was defined as the time from the diagnosis of BC to any type of relapse of the disease. DFS was defined as the interval between the initial primary diagnosis of BC and the first relapse or death. PFS was the duration from diagnosis until the first BC progression, death from any cause, or the final follow-up.

The endpoint/outcome measures extracted or calculated were the HRs and their 95% CIs for OS, RFS, DFS, PFS or BCSS, and the events/total events for age, tumor size, ER status, PR status, Her2 status, lymph node metastasis status, Ki67 expression, P53 expression, histological grade, distant metastasis and vascular invasion. Given that various studies have used different definitions for Tregs, we considered HR as the risk ratio between tumors with positive/rich Treg infiltration versus those with no/low Treg infiltration. HR estimates (with the corresponding 95% CIs) for a high density over a low density of Tregs and the HR cutoff point were obtained.

The most frequently used cutoff values for the high versus low/present versus absent density of Tregs were the median (n=11). The values were calculated using several semi-quantitative methods, including X-tile software (n=4) and others (n=3). We also conducted subgroup analyses to investigate the associations between prognostic outcomes (OS, BCSS, RFS, PFS and DFS) and Treg status in different ER-status BC patients (ER positive or ER negative).

Statistical analysis. For time-to-event outcomes, we used HRs and their 95% CIs to estimate the association between Tregs and prognosis. If the survival or mortality rate was not directly available, the 3-, 5-, and 10-year survival data extracted from Kaplan–Meier curves were read by the Engauge Digitizer version 4.1 (http://digitizer.sourceforge.net/) as described previously [23]. Two independent researchers performed the

Table 1. Pooled HR and 95% CI in meta-analysis of association of Treg infiltration (low vs high) with OS/BCSS or PFS/RFS in ER- negative patients

					Heterogeneity	
Outcome or Subgroup	Studies	Participants	Statistical Method	Effect Estimate	P	I^2(%)
OS/BCSS-3Y	4	1790	Risk Ratio (M-H, Random, 95% CI)	1.19 [0.80,1.77]	0.378	62.3
OS/BCSS-5Y	4	1790	Risk Ratio (M-H, Random, 95% CI)	1.28 [0.81, 2.04]	0.286	80.7
OS/BCSS-10Y	3	1298	Risk Ratio (M-H, Fixed, 95% CI)	1.05 [0.91, 1.22]	0.464	0.0
PFS/RFS-3Y	3	744	Risk Ratio (M-H, Random, 95% CI)	1.15 [0.41, 3.91]	0.794	93.8
PFS/RFS-5Y	3	744	Risk Ratio (M-H, Random, 95% CI)	0.73 [0.48, 1.12]	0.149	73.0
PFS/RFS-10Y	2	252	Risk Ratio (M-H, Random, 95% CI)	0.73 [0.37, 1.41]	0.342	84.4

process to reduce inaccuracies in the extracted survival rates (Y. W. and R. Z. Z.).

Inter-study heterogeneity was quantified using the I^2 statistic, with an I^2 value=50% indicating substantial heterogeneity, which was calculated as a pooled HR using the Mantel-Haenszel method for a random-effects model. We did not pool results for other outcomes given the small number of studies, heterogeneity between studies, or insufficient reporting. Reasons for statistical heterogeneity were explored through subgroup analysis, meta-regression or sensitivity analyses (when I^2 value>75%), the latter of which was also used to assess the impact of study quality. Publication bias was evaluated using Egger's plots and Begg's funnel plots because studies with positive results are more likely to be published than studies with negative results. All of the P-values were two-sided, and P<0.05 was considered statistically significant for all of the analyses, with the exception of publication bias, for which P<0.10 was considered significant [24]. All of the statistical calculations were performed using STATA 11.0 (StatCorp, College Station, TX, USA) and Revman 5.3 (Nordic Cochran Centre, Copenhagen, Denmark).

Results

Description of the studies. The flowchart for our search strategy is presented in Fig. 1.

Study characteristics and quality. The baseline characteristics of each study are summarized in Supplementary Table 2 (Bates et al. 2006 [16]; Ghebeh et al. 2008 [17]; Aruga et al.

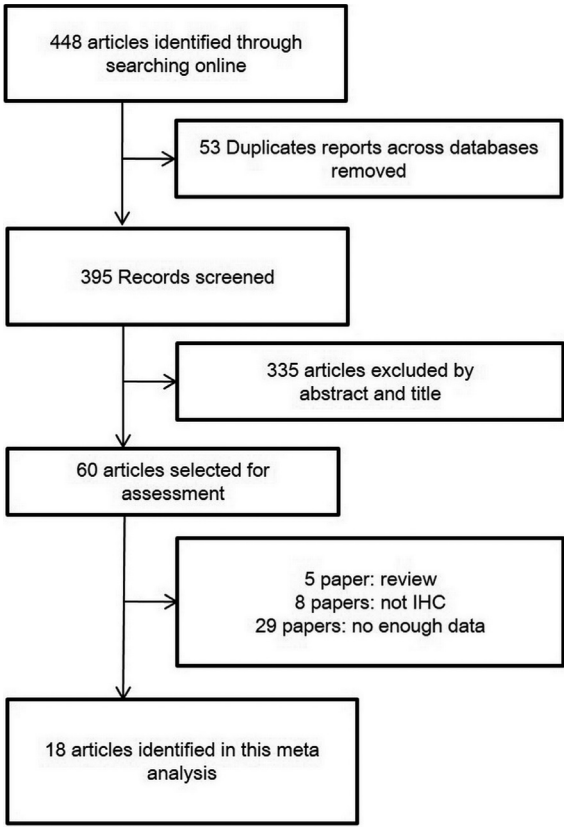

Figure 1. Selection of studies. Flow chart showing the selection process for the included studies.

Table 2. Pooled RR and 95% CI in meta-analysis of association of Treg infiltration with clinic-pathological factors

					Heterogeneity		
Outcome or Subgroup	Studies	Participants	Statistical Method	Effect Estimate	χ2	P	I^2(%)
Age(<50)	4	4147	Relative risk (M-H, Random, 95% CI)	0.87 [0.69,1.09]	9.15	0.226	67.2
Tumor size(≤ 2cm)	8	3580	Relative risk (M-H, Random, 95% CI)	1.06 [0.96, 1.19]	15.57	0.257	55.0
PR status (-)	9	3987	Relative risk (M-H, Random, 95% CI)	0.66 [0.51, 0.87]	97.01	0.003	91.8
ER status (-)	15	8268	Relative risk (M-H, Random, 95% CI)	0.54 [0.44, 0.66]	85.90	<0.001	83.7
Her2 status (-)	17	8370	Relative risk (M-H, Random, 95% CI)	1.08 [1.04, 1.12]	43.40	<0.001	63.1
Lymphatic metastasis (-)	12	6805	Relative risk (M-H, Random, 95% CI)	1.07 [0.96, 1.19]	35.20	0.217	68.7
Histological grade (I~II)	7	4453	Relative risk (M-H, Random, 95% CI)	1.35 [1.14, 1.59]	27.57	0.001	78.2
Ki67 (-)	4	990	Relative risk (M-H, Random, 95% CI)	1.43 [0.83, 2.47]	28.05	0.200	89.3
Distant metastasis (-)	2	1704	Relative risk (M-H, Random, 95% CI)	1.03 [0.90, 1.18]	13.01	0.670	92.3
P53 status (-)	2	314	Relative risk (M-H, Fixed, 95% CI)	1.10 [0.80, 1.51]	0.19	0.549	0
Vascular invasion (-)	4	4721	Relative risk (M-H, Fixed, 95% CI)	1.05 [1.00, 1.10]	1.11	0.069	0

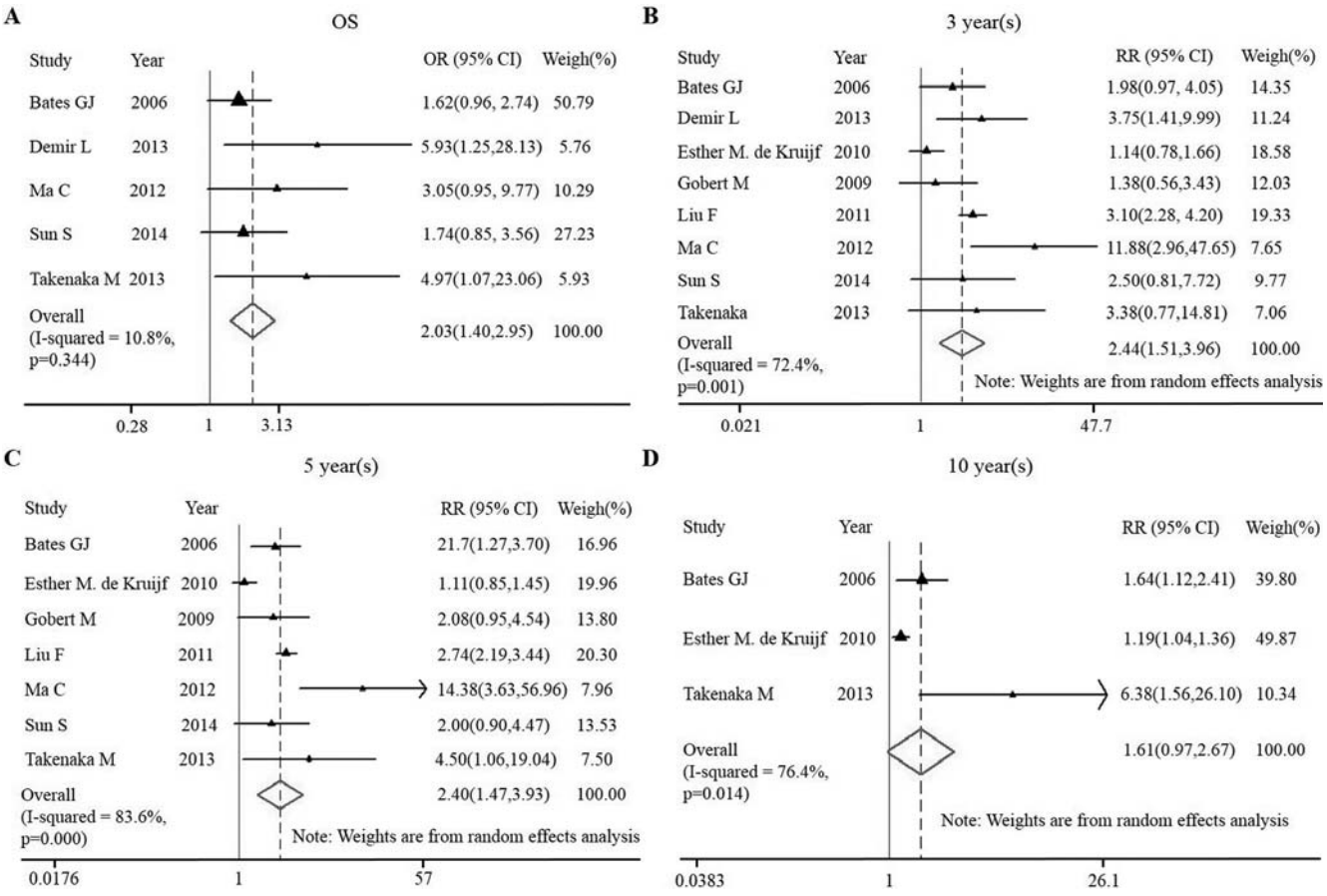

Figure 2. The forest plot of ORs for OS (A), as well as the RRs for the 3-year OS rate (B), 5-year OS rate (C), and 10-year OS rate (D) among the included studies.

2009 [25]; Gobert et al. 2009 [26]; de Kruijf et al. 2010 [27]; Liu et al. 2011 [18]; Mahmoud et al. 2011 [28]; Yan et al. 2011 [19]; Ma et al. 2012 [29]; Demir et al. 2013 [30]; Seo et al. 2013 [15]; Takenaka et al. 2013 [31]; Won et al. 2013 [32]; West et al. 2013 [23]; Kim et al. 2013 [13]; Liu et al. 2014 [20]; Maeda et al. 2014 [14]; Sun et al. 2014 [33]). The majority of the studies were performed in Asia (n=8). Other studies were conducted in Europe (n=7) and North America (n=3). The total sample size from all studies was 8,562, with a mean of 476 patients (ranging from 60 to 3,276 patients). All studies were published between 2006 and 2014. The qualities of all the eligible studies are presented in Supplementary Table 1. The median final score of the studies was 6.

Impact of Treg infiltration on OS and BCSS. The relationship between Treg infiltration and BC prognosis is illustrated in Fig. 2. HRs for OS were available in 5 studies. The pooled HR revealed a significantly increased risk of mortality in the high Treg group (OR, 2.03; 95% CI, 1.40–2.95; P=0.000 and I^2=10.80%, fixed effect; Fig. 2A). Then, we extracted overall survival data during follow-up at 3, 5, and 10 years after surgical resection from the survival curves of 8 articles. The overall mortality rate during the 3-year follow-up was significantly increased in the high Treg infiltration patients compared with the low Treg infiltration patients, with a combined RR of 2.44 (n=8; 95% CI, 1.51–3.96, P=0.000; Fig. 2B). Subgroup analysis revealed that the geographic region potentially caused the heterogeneity (data not shown). The overall mortality rate during the 5-year follow-up was significantly increased in the high Treg infiltration patients, with a combined risk ratio (RR) of 2.40 (n=7; 95% CI, 1.47–3.93, P=0.000; Fig. 2C). However, no statistically significant survival difference was noted at 10 years (95% CI, 0.97–2.67, P=0.065; Fig. 2D) possibly due to the small number of studies (Supplementary Table 3).

HRs for BSCC were available in only 3 studies. The pooled HR revealed a slightly increased risk of mortality in the patients with high Treg infiltration (OR, 1.05; 95% CI, 0.82–1.35; Fig. 3A), but this finding did not reach statistical significance (P=0.698). However, the mortality rate during the 3-, 5-, and 10-year follow-up was significantly increased in the high Treg infiltration patients (P<0.05; Fig. 3B-D; (Supplementary Table 4).

Impact of Treg infiltration on RFS/DFS/PFS. Given the relatively small number of studies with endpoints of PFS, RFS or DFS, we performed a pooled analysis of these three

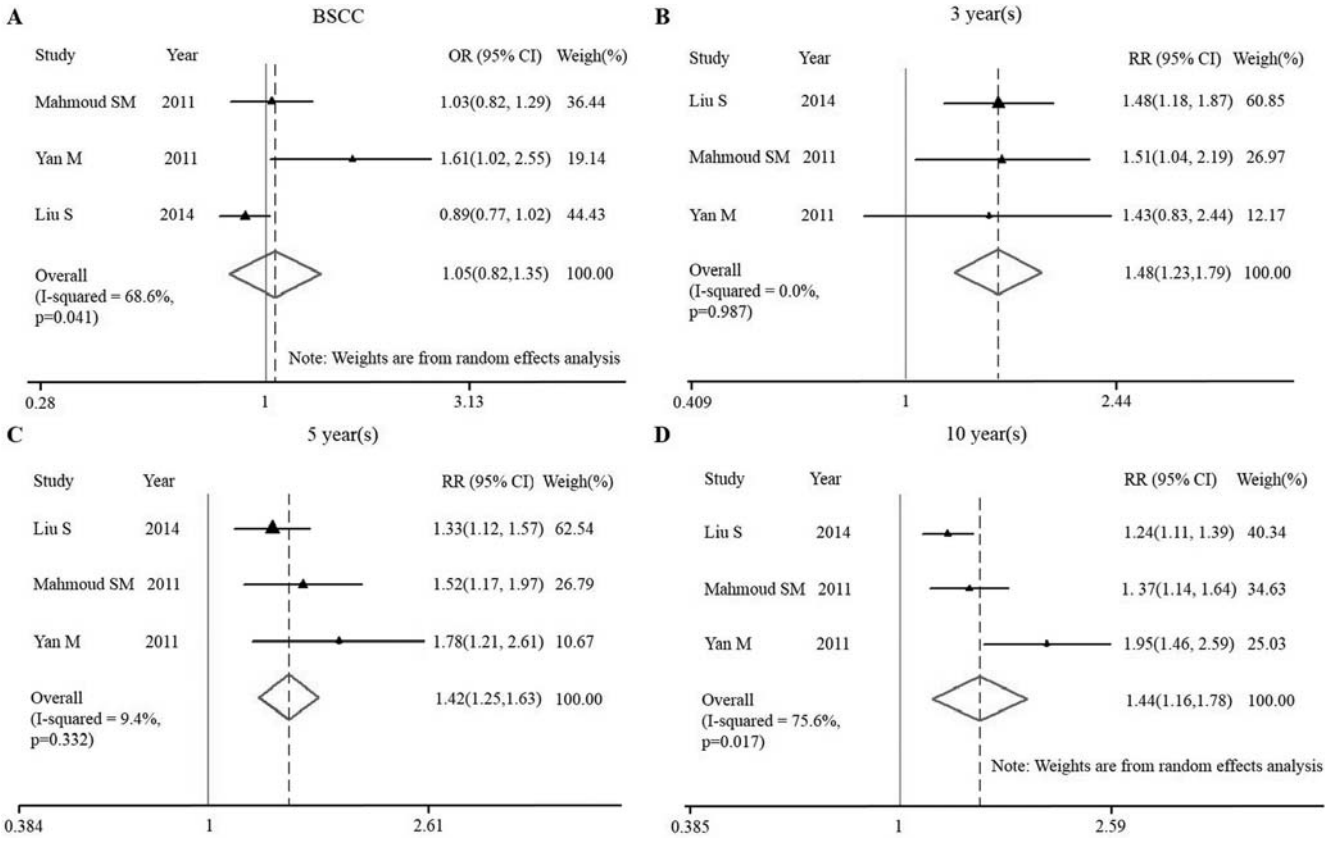

Figure 3. The forest plot of ORs for BSCC (A), as well as the RRs for the 3-year BSCC rate (B), 5-year BSCC rate (C), and 10-year BSCC rate (D) among the included studies.

indicators. HRs for RFS/DFS/PFS were available in 8 studies. The pooled HR exhibited a significantly increased risk of mortality in the high Treg group (OR, 1.48; 95% CI, 1.00–2.19; P=0.050; Fig. 4A).

The mortality rate during the 3-year follow-up period was significantly increased in the high Treg infiltration patients compared with the low Treg infiltration patients, with a combined RR of 1.93 (95% CI, 1.16–3.23, P=0.012; Fig. 4B). The mortality rate during the 5-year follow-up period was significantly increased in the high Treg infiltration patients, with a combined RR of 1.86 (95% CI, 1.64–2.11, P=0.000; Fig. 4C). Only one study provided data on 10-year RFS survival, so these data were not included in the meta-analysis [16] (Supplementary Table 5).

Meta-analysis based on ER status. Patients with different ER status were divided into subgroups. We pooled the analysis of the two end points OS and BCSS because relatively few studies reported this information.

In ER-positive patients, HRs for BSCC and OS were available in 4 studies. The pooled HR revealed that the risk of mortality did not significantly differ between the two Treg infiltration groups (OR, 1.38 1; 95% CI, 0.91–2.09, P=0.13). However, the BCSS/OS mortality rate at the 3-, 5-, and 10-year follow-up was significantly increased in the high Treg infiltration patients (3 years: RR, 2.04, 1; 95% CI, 1.16–3.61, P=0.014; 5 years: RR, 2.13, 1; 95% CI, 1.49–3.04, P=0.000; 10 years: RR, 1.38, 1; 95% CI, 1.22–1.57, P=0.000) (Supplementary Table 6).

HRs for RFS/DFS/PFS were available in 3 studies. The pooled HR revealed that the risk of mortality did not significantly differ between the Treg infiltration groups (OR, 2.16 1; 95% CI, 0.85–5.48, P=0.104). The RFS/DFS/PFS rate during the 3- or 5-year follow-up was significantly reduced in the high Treg infiltration patients compared with the low Treg infiltration patients (3 years: RR, 2.74; 95% CI, 1.40–5.35; 5 years: RR, 1.82; 95% CI, 1.14–2.89, P<0.05). Only one paper provided the 10-year RFS; thus, this statistic was not included in the meta-analysis [16] (Supplementary Table 7).

In ER-negative patients, we did not identify any significant difference between the Treg infiltration groups regarding the BCSS/OS rate or the RFS/DFS/PFS rate at 3, 5, or 10 years (Table 1; detailed in Supplementary Table 8 and Table 9).

Correlations of Treg infiltration with clinico-pathological parameters. To gain further insight into the value of Treg infiltration as an effective biomarker, we investigated the association between low Treg infiltration and various clinico-

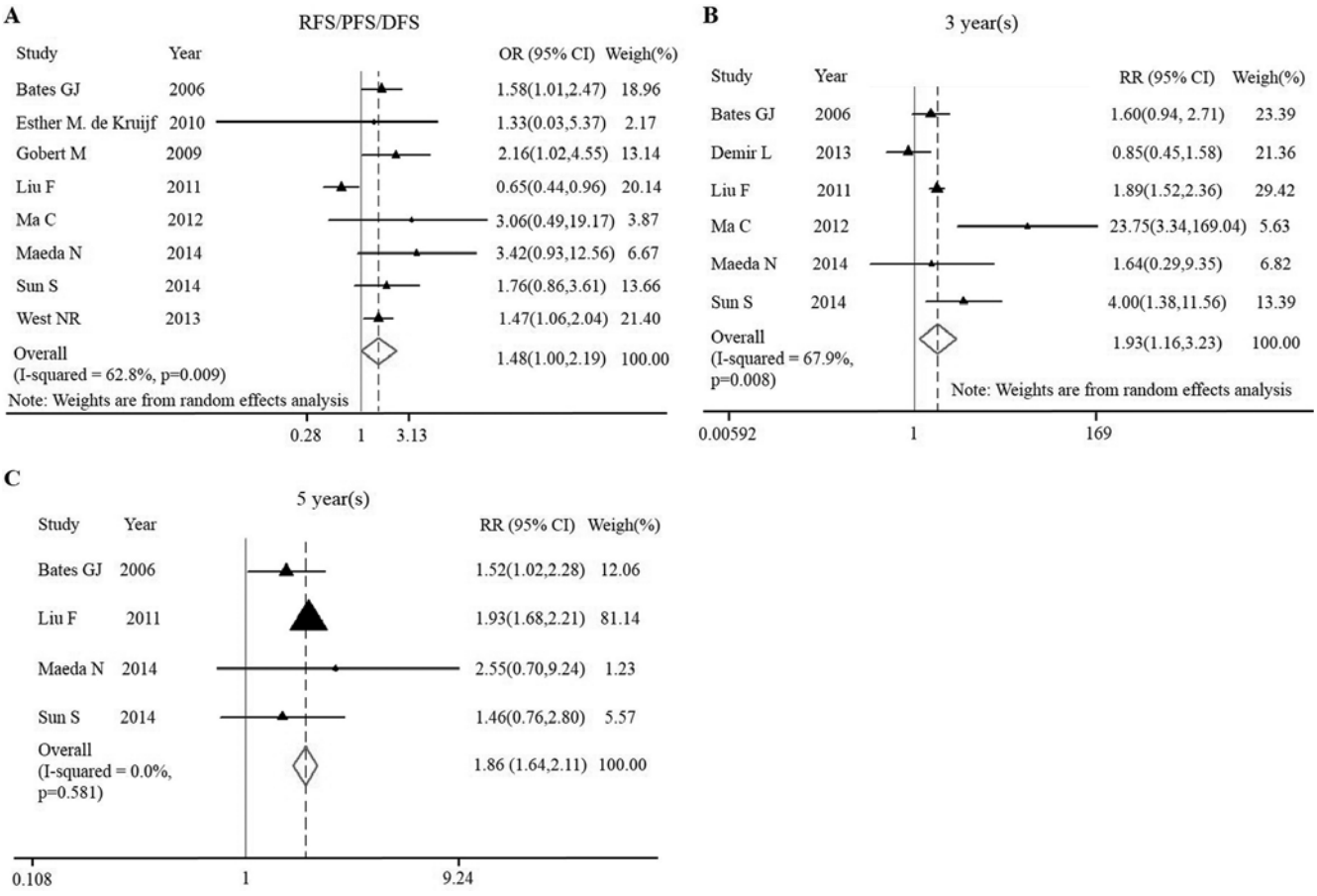

Figure 4. The forest plot of ORs for RFS/PFS/DFS (A) as well as the RRs for the 3-year RFS/PFS/DFS rate (B) and 5-year RFS/PFS/DFS rate (C) among the included studies.

pathological indicators (Table 2; detailed in Supplementary Table 10). Treg infiltration was not associated with specific clinical parameters for BC, such as age (<50, ≥50), tumor size (≤2 cm, >2 cm), Ki67 status (low, high), distant metastasis (negative, positive), P53 status, vascular invasion, or lymphatic metastasis. However, BCs with low Treg infiltration were positively associated with PR-positive status (pooled RR=0.66, 95% CI: 0.51-0.87, P=0.003 and I^2=91.8%, random effect), ER-positive status (pooled RR=0.54, 95% CI: 0.44-0.66, P=0.000 and I^2=83.7%, random effect), Her2-negative status (pooled RR=1.08, 95% CI: 1.04-1.12, P=0.000 and I^2=63.1%, random effect), and histological grade I or II (pooled RR=1.35, 95% CI: 1.14-1.59, P=0.001 and I^2=78.2%, random effect).

To investigate the observed heterogeneity (I^2>75%) in the analyses of clinico-pathological parameters, we performed a set of meta-regressions to determine to what extent the effects of clinical variables, such as publication year, continent (Asia, Europe or other), sample size (n≤200 or n>200) and cutoff method (median or other), could explain the heterogeneity of ER, PR and histological grade. The value of tau-squared decreased from 0.1082 to 0.0942 for ER status, 0.1352 to 0.06782 for PR status, and 0.03567 to 0.001641 for histological grade, which could partly explain the heterogeneity.

Sensitivity analysis and publication bias. To evaluate the influence of single studies on the pooled HRs, we performed a sensitivity analysis by estimating the average HR in the absence of each study. The results indicated that no individual studies significantly influenced the pooled HRs except that Liu et al. 2011 [18] influenced the DFS/RFS/PFS and 5-year DFS/RFS/PFS and Liu et al. 2014 [20] influenced the OS/BCSS and RFS/PFS in the ER_+ patients (data not shown).

Begg's funnel plot and Egger's linear regression test did not indicate evidence of an obvious publication bias, expect in the pooled analyses for OS (Egger's test: P=0.003; Begg's test: P=0.027), OS/BCSS (Egger's test: P=0.049; Begg's test: P=0.089) and 10-year OS/BCSS in the ER^+ patients (Egger's test: P=0.009; Begg's test: P=0.089) (Fig. 5).

Discussion

In recent years, the prognosis of patients with BC has improved remarkably, thanks to a variety of treatments, in-

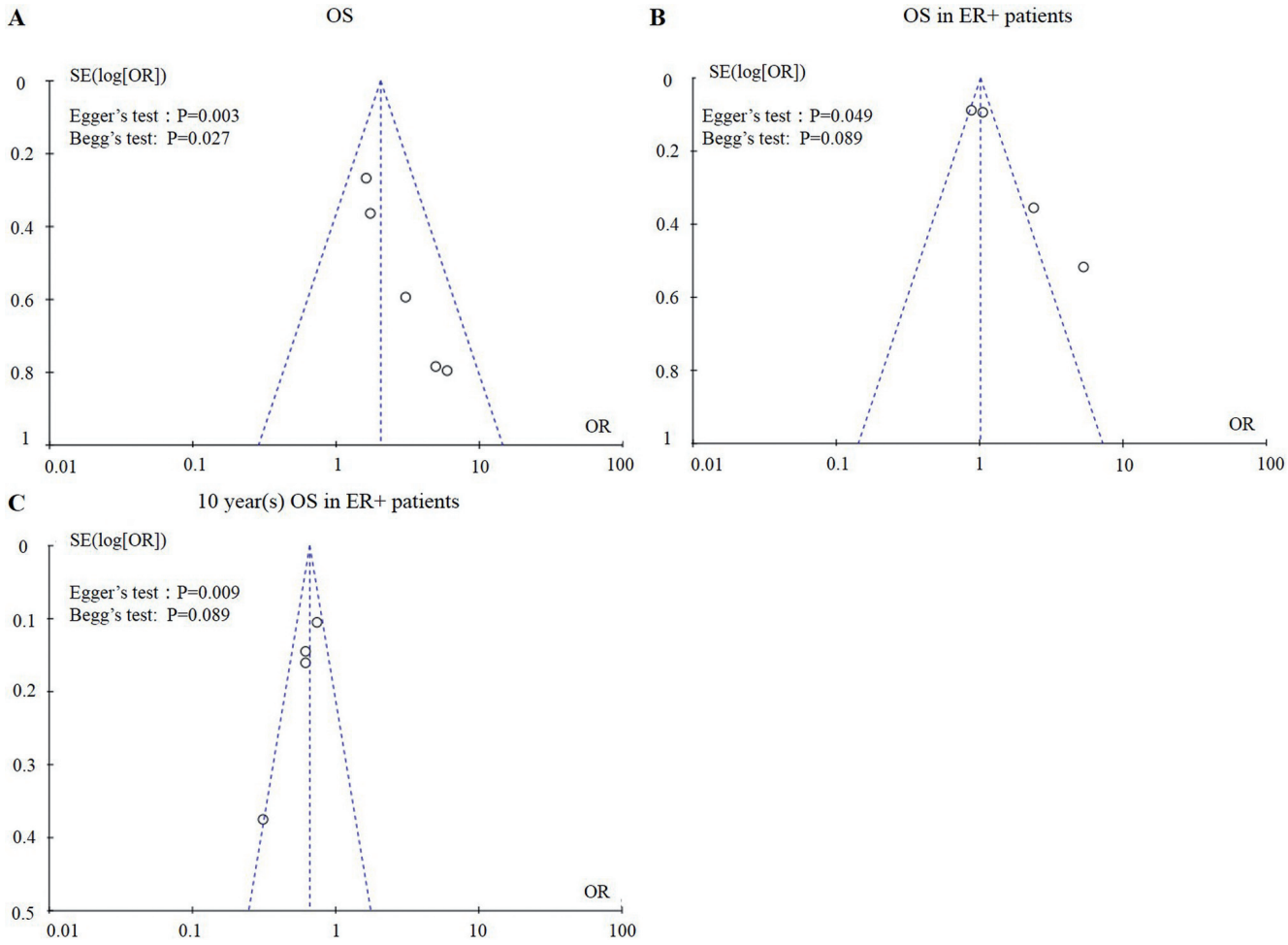

Figure 5. Publication bias in the meta-analysis. OS (A) in the included studies. OS (B) and 10-year OS (C) in the ER-positive BC patients.

cluding surgical resection, adjuvant chemotherapy, hormonal therapy, and radiation therapy. However, the problems of recurrence, metastasis, and drug resistance have not been resolved, and the causes and mechanisms of the above phenomena have not been clarified. Although several risk factors for the development of BC have been evaluated, this is a field of ongoing investigation. Treg infiltration is a known risk factor for cancer incidence and mortality. This study is the first comprehensive meta-analysis to determine the significance of Treg infiltration in BC. The current literature provides a definitive association between Treg infiltration and outcome and clinico-pathological factors of BC. Our study revealed several points worth discussing.

First, our study confirmed the prognostic value of Treg infiltration in BC on OS, PFS/DFS/RFS and BCSS. Patients with Treg infiltration exhibited poorer OS, PFS/DFS/RFS or BCSS compared with patients who lacked Tregs. How does Treg infiltration account for the poor prognosis in BC? Treg cells are found at high concentrations in various types of tumor tissues, such as the lung, liver, pancreas, gastrointestinal tract, BC and malignant melanoma and are associated with poor prognosis in ovarian, breast, and gastric cancers [34]. Tregs in cancer patients can recognize a broad range of tumor antigens, including survivin and NY-ESO-1, and can suppress tumor antigen-specific T cells [35]. Emerging evidence suggests that Tregs may exhibit specialized functions that affect both angiogenesis and metastasis within the tumor environment. Under hypoxic conditions, Tregs produce vascular endothelial growth factor A (VEGFA), promoting the differentiation of endothelial cells and inducing angiogenesis [36]. Tregs also play a role in BC metastasis by expressing RANK ligand (RANKL) [37]. A growing number of chemokine-receptor axes, including CCL22 and its receptor CCR4 [38], have been implicated in the trafficking of Tregs to different types of cancer [39]. CXCR4 and its chemokine ligand 12 (CXCL12) are two key factors in BC metastasis [40]. Therefore, Tregs could be a marker for poor BC prognosis.

In our study, Treg infiltration was linked to ER status, PR status, Her2 status and histological grade. Tregs are more prone to infiltrate in BC patients with ER negativity, PR negativity,

HER2 positivity and histological grade III. ER, PR, and HER2/neu are the most important tissue markers used in the management of BC in the adjuvant treatment and metastatic disease settings. ER-positive BCs are associated with good outcomes and histology. In breast tumors, the ER participates in tumor biology and recurrence patterns [41]. Furthermore, loss of PR expression potentially has an important prognostic effect because ER-positive/PR-negative tumors are more aggressive and associated with a reduced OS rate compared with ER- and PR-positive tumors [42]. Histological grade and Her2 are also prognostic factors for BC [43, 44], and Her2 overexpression is correlated with increased cell proliferation and motility, angiogenesis and tumor invasiveness.

Considering the importance of ER status for prognosis of BC, we performed subgroup analysis according to ER status. Among ER-positive BC patients, low Treg infiltration patients exhibited a higher survival rate at 3-, 5-, and 10-year follow-up, but the Cox regression did not reveal any difference. However, among ER-negative BC patients, we observed no significant correlation between Treg infiltration and prognosis, which may have been attributed to the small number of relevant studies included in this analysis.

Our study has several strengths. First, our study is large and is the only study to date to evaluate the association between Treg infiltration and BC outcome. Second, the majority of the studies were of high quality. Third, study-level data for ER status, PR status, Her2 status, histopathological differentiation and tumor size allowed meaningful subset analyses. However, our study also has some limitations. First, the number of included studies was relatively small. Because these 8,562 patients exhibited different TMN stages and received various follow-up treatments, we were unable to assess the potential outliers present in individual studies. Second, the cutoff values differed between studies. The median of molecular marker levels was used as the cutoff between high and low or present and absent in 9 studies. In addition, other studies used different cutoffs, which could have caused heterogeneity among the studies. Third, Treg infiltration in the included studies was measured primarily using IHC, the results of which are strongly dependent upon methodological factors, such as primary antibody and secondary antibody concentrations. Finally, the studies included in this meta-analysis were from Asia, Europe and North America. Distinct location or race differences are believed to exist and could cause publication bias. We could not perform subgroup analysis for survival to explore this influence because few studies offered concrete data.

In conclusion, this meta-analysis indicates a positive association between Treg infiltration and poor outcomes in BC. To make Tregs clinically useful in BC, particularly for prognosis, additional large prospective studies should be conducted.

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Supplementary information is available in the online version of the paper.

References

[1] SIEGEL RL, MILLER KD, JEMAL A. Cancer statistics, 2015. CA Cancer J Clin 2015; 65: 5–29. http://dx.doi.org/10.3322/caac.21254

[2] MELLMAN I, COUKOS G, DRANOFF G. Cancer immunotherapy comes of age. Nature 2011; 480: 480–489. http://dx.doi.org/10.1038/nature10673

[3] STANTON SE, DISIS ML. Designing vaccines to prevent breast cancer recurrence or invasive disease. Immunotherapy 2015; 7: 69–72. http://dx.doi.org/10.2217/imt.15.5

[4] CONVIT J, MONTESINOS H, OVIEDO H, ROMERO G, MACCARONE B et al. Autologous tumor lysate/bacillus Calmette-Guerin immunotherapy as an adjuvant to conventional breast cancer therapy. Clin Transl Oncol 2015; 17: 884–887. http://dx.doi.org/10.1007/s12094-015-1320-0

[5] SHEVACH EM. CD4+ CD25+ suppressor T cells: more questions than answers. Nat Rev Immunol 2002; 2: 389–400. doi: 10.1038/nri821.

[6] LIOTTA LA, KOHN EC. The microenvironment of the tumour-host interface. Nature 2001; 411: 375–379. http://dx.doi.org/10.1038/35077241

[7] LI H, FAN X, HOUGHTON J. Tumor microenvironment: the role of the tumor stroma in cancer. J Cell Biochem 2007; 101: 805–815. http://dx.doi.org/10.1002/jcb.21159

[8] ZHAO HQ, LI WM, LU ZQ, YAO YM. Roles of Tregs in development of hepatocellular carcinoma: a meta-analysis. World J Gastroenterol 2014; 20: 7971–7978. http://dx.doi.org/10.3748/wjg.v20.i24.7971

[9] HUANG Y, LIAO H, ZHANG Y, YUAN R, WANG F et al. Prognostic value of tumor-infiltrating FoxP3+ T cells in gastrointestinal cancers: a meta analysis. PLOS ONE 2014; 9: e94376. http://dx.doi.org/10.1371/journal.pone.0094376

[10] DELEEUW RJ, KOST SE, KAKAL JA, NELSON BH. The prognostic value of FoxP3+ tumor-infiltrating lymphocytes in cancer: a critical review of the literature. Clin Cancer Res 2012; 18: 3022–3029. http://dx.doi.org/10.1158/1078-0432.CCR-11-3216

[11] LADOIRE S, MARTIN F, GHIRINGHELLI F. Prognostic role of FOXP3+ regulatory T cells infiltrating human carcinomas: the paradox of colorectal cancer. Cancer Immunol Immunother 2011; 60: 909–918. http://dx.doi.org/10.1007/s00262-011-1046-y

[12] SALAMA P, PHILLIPS M, GRIEU F, MORRIS M, ZEPS N et al. Tumor-infiltrating FOXP3+ T regulatory cells show strong prognostic significance in colorectal cancer. J Clin Oncol 2009; 27: 186–192. http://dx.doi.org/10.1200/JCO.2008.18.7229

[13] KIM ST, JEONG H, WOO OH, SEO JH, KIM A et al. Tumor-infiltrating lymphocytes, tumor characteristics, and recurrence in patients with early breast cancer. Am J Clin Oncol 2013; 36: 224–231. http://dx.doi.org/10.1097/COC.0b013e3182467d90

[14] MAEDA N, YOSHIMURA K, YAMAMOTO S, KURAMASU A, INOUE M et al. Expression of B7-H3, a potential factor of tumor immune evasion in combination with the number of regulatory T cells, affects against recurrence-free survival in breast cancer patients. Ann Surg Oncol 2014; 21 (Suppl 4): S546-S554. http://dx.doi.org/10.1245/s10434-014-3564-2

[15] SEO AN, LEE HJ, KIM EJ, KIM HJ, JANG MH et al. Tumour-infiltrating CD8+ lymphocytes as an independent predictive factor for pathological complete response to primary systemic therapy in breast cancer. Br J Cancer 2013; 109: 2705–2713. http://dx.doi.org/10.1038/bjc.2013.634

[16] BATES GJ, FOX SB, HAN C, LEEK RD, GARCIA JF et al. Quantification of regulatory T cells enables the identification of high-risk breast cancer patients and those at risk of late relapse. J Clin Oncol 2006; 24: 5373–5380. http://dx.doi.org/10.1200/JCO.2006.05.9584

[17] GHEBEH H, BARHOUSH E, TULBAH A, ELKUM N, AL-TWEIGERI T et al. FOXP3+ Tregs and B7-H1+/PD-1+ T lymphocytes co-infiltrate the tumor tissues of high-risk breast cancer patients: implication for immunotherapy. BMC Cancer 2008; 8: 57. http://dx.doi.org/10.1186/1471-2407-8-57

[18] LIU F, LANG R, ZHAO J, ZHANG X, PRINGLE GA et al. CD8+ cytotoxic T cell and FOXP3+ regulatory T cell infiltration in relation to breast cancer survival and molecular subtypes. Breast Cancer Res Treat 2011; 130: 645–655. http://dx.doi.org/10.1007/s10549-011-1647-3

[19] YAN M, JENE N, BYRNE D, MILLAR EK, O'TOOLE SA et al. Recruitment of regulatory T cells is correlated with hypoxia-induced CXCR4 expression, and is associated with poor prognosis in basal-like breast cancers. Breast Cancer Res 2011; 13: R47. http://dx.doi.org/10.1186/bcr2869

[20] LIU S, FOULKES WD, LEUNG S, GAO D, LAU S et al. Prognostic significance of FOXP3+ tumor-infiltrating lymphocytes in breast cancer depends on estrogen receptor and human epidermal growth factor receptor-2 expression status and concurrent cytotoxic T-cell infiltration. Breast Cancer Res 2014; 16: 432. http://dx.doi.org/10.1186/s13058-014-0432-8

[21] HAYES DF, BAST RC, DESCH CE, FRITSCHE HJR, KEMENY NE et al. Tumor marker utility grading system: a framework to evaluate clinical utility of tumor markers. J Natl Cancer Inst 1996; 88: 1456–1466. http://dx.doi.org/10.1093/jnci/88.20.1456

[22] MCSHANE LM, ALTMAN DG, SAUERBREI W, TAUBE SE, GION M et al. REporting recommendations for tumour MARKer prognostic studies (REMARK). Br J Cancer 2005; 93: 387–391. http://dx.doi.org/10.1038/sj.bjc.6602678

[23] WEST NR, KOST SE, MARTIN SD, MILNE K, DELEEUW RJ et al. Tumour-infiltrating FOXP3(+) lymphocytes are associated with cytotoxic immune responses and good clinical outcome in oestrogen receptor-negative breast cancer. Br J Cancer 2013; 108: 155–162. http://dx.doi.org/10.1038/bjc.2012.524

[24] HIGGINS JP, THOMPSON SG. Quantifying heterogeneity in a meta-analysis. Statist Med 2002; 21: 1539–1558. http://dx.doi.org/10.1002/sim.1186

[25] ARUGA T, SUZUKI E, SAJI S, HORIGUCHI S, HORIGUCHI K et al. A low number of tumor-infiltrating FOXP3-positive cells during primary systemic chemotherapy correlates with favorable anti-tumor response in patients with breast cancer. Oncol Rep 2009; 22: 273–278. http://dx.doi.org/10.3892/or_00000434

[26] GOBERT M, TREILLEUX I, BENDRISS-VERMARE N, BACHELOT T, GODDARD-LEON S et al. Regulatory T cells recruited through CCL22/CCR4 are selectively activated in lymphoid infiltrates surrounding primary breast tumors and lead to an adverse clinical outcome. Cancer Res 2009; 69 : 2000–2009. http://dx.doi.org/10.1158/0008-5472.CAN-08-2360

[27] DE KRUIJF EM, VAN NES JGH, SAJET A, TUMMERS QRJG, PUTTER H et al. The predictive value of HLA class I tumor cell expression and presence of intratumoral Tregs for chemotherapy in patients with early breast cancer. Clin Cancer Res 2010; 16: 1272–1280. http://dx.doi.org/10.1158/1078-0432.CCR-09-1844

[28] MAHMOUD SM, PAISH EC, POWE DG, MACMILLAN RD, LEE AH et al. An evaluation of the clinical significance of FOXP3+ infiltrating cells in human breast cancer. Breast Cancer Res Treat 2011; 127: 99–108. http://dx.doi.org/10.1007/s10549-010-0987-8

[29] MA C, ZHANG Q, YE J, WANG F, ZHANG Y et al. Tumor-infiltrating gamma delta T lymphocytes predict clinical outcome in human breast cancer. J Immunol 2012; 189: 5029–5036. http://dx.doi.org/10.4049/jimmunol.1201892

[30] DEMIR L, YIGIT S, ELLIDOKUZ H, ERTEN C, SOMALI I et al. Predictive and prognostic factors in locally advanced breast cancer: effect of intratumoral FOXP3+ Tregs. Clin Exp Metastasis 2013; 30: 1047–1062. http://dx.doi.org/10.1007/s10585-013-9602-9

[31] TAKENAKA M, SEKI N, TOH U, HATTORI S, KAWAHARA A. FOXP3 expression in tumor cells and tumor-infiltrating lymphocytes is associated with breast cancer prognosis. Molecular and Clinical Oncology 2013; 1: 625–632. http://dx.doi.org/10.3892/mco.2013.107

[32] WON KY, KIM HS, SUNG JY, KIM GY, LEE J et al. Tumoral FOXP3 has potential oncogenic function in conjunction with the p53 tumor suppressor protein and infiltrated Tregs in human breast carcinomas. Pathol Res Pract 2013; 209: 767–773. http://dx.doi.org/10.1016/j.prp.2013.08.010

[33] SUN S, FEI X, MAO Y, WANG X, GARFIELD DH et al. PD-1(+) immune cell infiltration inversely correlates with survival of operable breast cancer patients. Cancer Immunol Immunother 2014; 63: 395–406. http://dx.doi.org/10.1007/s00262-014-1519-x

[34] NISHIKAWA H, SAKAGUCHI S. Regulatory T cells in tumor immunity. Int J Cancer 2010; 127: 759–767. http://dx.doi.org/10.1002/ijc.25429

[35] LEHE C, GHEBEH H, AL-SULAIMAN A, AL QUDAIHI G, AL-HUSSEIN K et al. The Wilms' tumor antigen is a novel target for human CD4+ regulatory T cells: implications for immunotherapy. Cancer Res 2008; 68: 6350–6359. http://dx.doi.org/10.1158/0008-5472.CAN-08-0050

[36] FACCIABENE A, PENG X, HAGEMANN IS, BALINT K, BARCHETTI A et al. Tumour hypoxia promotes tolerance and angiogenesis via CCL28 and T(reg) cells. Nature 2011; 475: 226–230. http://dx.doi.org/10.1038/nature10169

[37] TAN W, ZHANG W, STRASNER A, GRIVENNIKOV S, CHENG JQ et al. Tumour-infiltrating regulatory T cells stimulate mammary cancer metastasis through RANKL-RANK signalling. Nature 2011; 470: 548–553. http://dx.doi.org/10.1038/nature09707

[38] CURIEL TJ, COUKOS G, ZOU L, ALVAREZ X, CHENG P et al. Specific recruitment of regulatory T cells in ovarian carcinoma fosters immune privilege and predicts reduced survival. Nat Med 2004; 10: 942–949. http://dx.doi.org/10.1038/nm1093

[39] MAILLOUX AW, YOUNG MR. Regulatory T-cell trafficking: from thymic development to tumor-induced immune suppression. Crit Rev Immunol 2010; 30: 435–447. http://dx.doi.org/10.1615/CritRevImmunol.v30.i5.30

[40] WENDEL C, HEMPING-BOVENKERK A, KRASNYANSKA J, MEES ST, KOCHETKOVA M et al. CXCR4/CXCL12 participate in extravasation of metastasizing breast cancer cells within the liver in a rat model. PLOS ONE 2012; 7: e30046. http://dx.doi.org/10.1371/journal.pone.0030046

[41] CADOO KA, FORNIER MN, MORRIS PG. Biological subtypes of breast cancer: current concepts and implications for recurrence patterns. Q J Nucl Med Mol Imaging 2013; 57: 312–321.

[42] BROOM RJ, TANG PA, SIMMONS C, BORDELEAU L, MULLIGAN AM et al. Changes in estrogen receptor, progesterone receptor and Her-2/neu status with time: discordance rates between primary and metastatic breast cancer. Anticancer Res 2009; 29: 1557–1562.

[43] ELSTON CW, ELLIS IO. Pathological prognostic factors in breast cancer. I. The value of histological grade in breast cancer: experience from a large study with long-term follow-up. Histopathology 1991; 19: 403–410. http://dx.doi.org/10.1111/j.1365-2559.1991.tb00229.x

[44] WOLFF AC, HAMMOND MEH, HICKS DG, DOWSETT M, MCSHANE LM et al. Recommendations for human epidermal growth factor receptor 2 testing in breast cancer: American Society of Clinical Oncology/College of American Pathologists clinical practice guideline update. J Clin Oncol 2013; 31: 3997–4013. http://dx.doi.org/10.1200/JCO.2013.50.9984

Supplementary Table 1 Assessment of methodological quality for each cohort study

Study	Is the population under study defined with in- and exclusion criteria?	Were patient data prospectively collected?	Are the main prognostic patient and tumor characteristics presented?[1]	Is the method used for determination of protein expression specified?	Is the IHC or HE staining protocol specified? [2]	Were stainings evaluated by > 1 observer?	Is the study endpoint defined?	Is the time of follow up specified?	Is loss during analysis or follow up described?	Quality rating
Bates et al.(2006)	1	0	1	1	1	0	1	1	0	6
Demir et al.(2013)	0	0	1	1	1	0	1	1	0	5
Esther M de Kruijf et al.(2010)	1	0	1	1	1	1	1	1	0	7
Liu et al.(2014)	0	0	1	1	1	1	1	1	0	6
Liu et al.(2011)	0	0	1	1	1	1	1	1	0	6
Mahmoud SM et al.(2011)	0	0	1	1	1	1	1	1	0	6

Gobert M et al.(2009)	0	0	1	1	1	1	0	1	0	5
Maeda N et al.(2014)	0	0	1	1	1	1	1	1	0	6
West et al.(2013)	1	0	1	1	1	1	1	1	0	7
Sun et al.(2014)	0	0	1	1	1	1	1	1	0	6
Takenaka M et al.(2013)	0	0	1	1	1	1	1	0	0	5
Ma et al.(2012)	0	1	1	1	1	1	1	0	0	6
Kim et al.(2013)	0	0	1	1	1	1	1	1	0	6
Ghebeh et al.(2005)	0	0	1	1	1	Not clear	0	0	0	3
Seo et al.(2013)	0	0	1	1	1	0	1	0	0	4
Yan M et al.(2011)	0	0	1	1	1	Not clear	0	1	0	4
Won KY et al.(2013)	0	0	1	1	1	1	0	1	0	5
Aruga T et al.(2009)	0	0	1	1	1	Not clear	0	1	0	4

Supplementary Table 2 Main characteristics of studies included in the meta-analysis

Study (year)	Country	Type of patients	Total(high/low)	Quality score	Primary endpoint	Marker	Survival analysis	Cutoff point
Bates GJ (2006)	United Kingdom	Breast Cancer	237（118/119)	6	RFS, OS	Foxp3	Multivariate; Kaplan-Meier	Median
Demir L (2013)	Turkey	LABC receive NAC	60(30/30)	5	RFS, OS	Foxp3	Multivariate; Kaplan-Meier	Median
Esther M. de Kruijf (2010)	The Netherlands	Non-metastasied BC	559(239/320)	7	RFS, OS	Foxp3	Multivariate; Kaplan-Meier	Median
Liu S (2014)	Canada	Invasive BC	3276(1031/2245)	6	BCSS, RFS	Foxp3	Multivariate; Kaplan-Meier	Median
Liu F (2011)	China	Invasive BC	1270(646/624)	6	PFS, OS	Foxp3	Multivariate; Kaplan-Meier	Median

Mahmoud SM (2011)	UK	Invasive BC	1190(322/868)	6	BCSS	Foxp3	Multivariate; Kaplan-Meier	X-tile software program high：≥28 in lymphoid area and≥18 in tumor area
Gobert M (2009)	France	Invasive metastasied BC	191(62/129)	5	OS	Foxp3	Multivariate; Kaplan-Meier	
Maeda N (2014)	Janpan	Invasive BC	90(43/47)	6	RFS, OS	Foxp3	Multivariate; Kaplan-Meier	Median
West NR (2013)	Canada	ER-BC	175(83/92)	7	BCSS, RFS	Foxp3	Multivariate; Kaplan-Meier	X-Tile software
Sun S (2014)	China	Invasive BC	208(104/104)	6	PFS, OS	Foxp3	Multivariate; Kaplan-Meier	Median
Yan M (2011)	UK,Australia	Invasive BC	479(217/262)	3	BCSS	Foxp3	Multivariate;	High: ≥15 Treg

							Kaplan-Meier	
Takenaka M(2013)	Janpan	Invasive BC	100(43/57)	5	RFS, OS	Foxp3	Multivariate; Kaplan-Meier	Median
Ma C (2012)	USA	primary breast cancer	81(36/45)	6	RFS, OS	Foxp3	Multivariate; Kaplan-Meier	Median
Kim ST (2013)	Korea	early breast cancer	72/	6	DFS	Foxp3	Multivariate; Kaplan-Meier	High：>17/hpf Low：≤17/hpf
Ghebeh H (2008)	Saudi Arabia	Breast Cancer	62(42/20)	3	/	Foxp3	/	Tregs:++ or +++
Won KY (2013)	South Korea	Breast Cancer	272(50/222)	5	DFS	Foxp3	Kaplan-Meier	High: ≥15 Treg
Seo AN (2013)	Korea	II or III breast cancer	153(79/74)	4	pCR	Foxp3	/	Median
Aruga T (2009)	Janpan	invasive breast carcinoma	87(43/44)	4	RFS, OS	Foxp3	Multivariate; Kaplan-Meier	Median

Supplementary Table 3 Detailed data in meta-analysis of association of Treg infiltration with OS in BC patients

			High		Low	
OS	No. of studies	Studies involved	Alive	Total	Alive	Total
3-year	8	Bates GJ (2006)	99	119	108	118
		Demir L (2013)	15	30	26	30
		Esther M. de Kruijf (2010)	197	239	268	317
		Gobert M (2009)	40	46	125	138
		Liu F（2011）	492	646	576	624
		Ma C（2012）	17	36	43	45
		Sun S（2014）	94	104	100	104
		Takenaka M（2013）	47	56	40	42
5-year	7	Bates GJ (2006)	84	119	102	118
		Esther M. de Kruijf (2010)	168	239	232	317
		Gobert M (2009)	37	46	125	138
		Liu F（2011）	413	646	542	624
		Ma C（2012）	13	36	43	45
		Sun S（2014）	88	104	96	104
		Takenaka M（2013）	44	56	40	42
10-year	3	Bates GJ (2006)	71	119	89	118
		Esther M. de Kruijf (2010)	79	239	139	317
		Takenaka M（2013）	39	56	40	42

Supplementary Table 4 Detailed data in meta-analysis of association of Treg infiltration with BCSS in BC patients

			High		Low	
BCSS	No. of studies	Studies involved	Alive	Total	Alive	Total
3-year	3	Liu S（2014）	920	1028	2074	2232
		Mahmoud SM（2011）	678	773	383	417
		Yan M（2011）	191	217	240	262
5-year	3	Liu S（2014）	843	1028	1929	2232
		Mahmoud SM（2011）	596	773	354	417
		Yan M（2011）	164	217	226	262
10-year	3	Bates GJ(2006)	71	119	89	118
		Esther M. de Kruijf(2010)	79	239	139	317
		Takenaka M（2013）	39	56	40	42

Supplementary Table 5 Detailed data in meta-analysis of association of Treg infiltration with RFS/PFS/DFS in BC patients

			High		Low	
RFS/PFS/DFS	No. of studies	Studies involved	Alive	Total	Alive	Total
3-year	6	Bates GJ（2006）	90	119	100	118
		Demir L (2013)	19	30	17	30
		Liu F（2011）	460	646	529	624
		Ma C（2012）	17	36	44	45
		Maeda N(2014)	40	43	45	47
		Sun S（2014）	88	104	100	104
5-year	4	Bates GJ(2006)	76	119	90	118
		Liu F（2011）	275	646	438	624
		Maeda N(2014)	36	43	44	47
		Sun S（2014）	85	104	91	104

Supplementary Table 6 Detailed data in meta-analysis of association of Treg infiltration with OS/BCSS in ER+ BC patients

OS/BCSS	No. of studies	Studies involved	High Alive	High Total	Low Alive	Low Total
3-year	6	Bates GJ（2006）	57	60	85	88
		Gobert M（2009）	37	38	98	103
		Liu F（2011）	218	272	480	506
		Liu S（2014）	603	640	1647	1711
		Ma C（2012）	12	25	30	33
		Mahmoud SM（2011）	437	471	304	323
5-year	6	Bates GJ（2006）	46	60	80	88
		Gobert M（2009）	33	38	96	103
		Liu F（2011）	183	272	446	506
		Ma C（2012）	9	25	30	33
		Mahmoud SM（2011）	385	471	283	323
		Liu S（2014）	555	640	1563	1711
10-year	3	Bates GJ（2006）	34	60	71	88
		Liu S（2014）	463	640	1335	1711
		Mahmoud SM（2011）	307	471	243	323

Supplementary Table 7 Detailed data in meta-analysis of association of Treg infiltration with PFS/DFS in ER+ BC patients

			High		Low	
PFS/DFS	No. of studies	Studies involved	Alive	Total	Alive	Total
3-year	3	Bates GJ（2006）	48	60	79	88
		Liu F（2011）	191	272	444	506
		Ma C（2012）	12	25	32	33
5-year	3	Bates GJ（2006）	42	60	68	88
		Liu F（2011）	157	272	377	506
		West NR(2013)	11	25	29	33

Supplementary Table 8 Detailed data in meta-analysis of association of Treg infiltration with OS/BCSS in ER- BC patients

			High		Low	
OS/BCSS	No. of studies	Studies involved	Alive	Total	Alive	Total
3-year	4	Bates GJ（2006）	41	50	20	27
		Liu F（2011）	264	374	101	118
		Liu S（2014）	317	388	427	521
		Mahmoud SM（2011）	188	256	43	56
5-year	4	Bates GJ（2006）	34	50	21	27
		Liu F（2011）	232	374	97	118
		Liu S（2014）	288	388	366	521
		Mahmoud SM（2011）	162	256	38	56
10-year	3	Bates GJ（2006）	17	50	11	27
		Liu S（2014）	241	388	330	521
		Mahmoud SM（2011）	139	256	33	56

Supplementary Table 9 Detailed data in meta-analysis of association of Treg infiltration with RFS/DFS/PFS in ER- BC patients

			High		Low	
RFS/DFS/PFS	No. of studies	Studies involved	Alive	Total	Alive	Total
3-year	3	Bates GJ（2006）	34	50	18	27
		Liu F（2011）	188	374	95	118
		West NR(2013)	61	92	37	83
5-year	3	Bates GJ（2006）	25	50	17	27
		Liu F（2011）	300	374	76	118
		West NR(2013)	58	92	34	83
10-year	2	Bates GJ（2006）	23	50	13	27
		West NR(2013)	55	92	20	83

Supplementary Table 10 Detailed data in meta-analysis of association of Treg infiltration with clinic-pathological factors

Outcome/Subgroup	Participants	Studies involved	No. of patients	
			Low	High
Age (<50/≥50)	4147	Aruga T(2009)	20/23	19/25
		Bates GJ(2006)	32/83	34/76
		Esther M. de Kruijf(2010)	108/212	82/157
		Liu S(2014)	570/1675	370/661
Tumor size(≤2cm/2cm)	3580	Bates GJ (2006)	60/55	56/54
		Gobert M (2009)	109/20	56/6
		Liu F (2011)	164/440	167/479
		Ma C (2012)	27/17	14/22
		Maeda N (2014)	28/19	12/31
		Mahmoud SM (2011)	341/177	563/355
		Sun S (2014)	51/53	53/51
		Takenaka M (2013)	26/17	32/25
PR status(-/+)	3987	Aruga T(2009)	12/31	29/15
		Demir L(2013)	11/19	14/16
		Esther M. de Kruijf(2010)	116/183	100/121
		Ghebeh H(2008)	7/13	25/17
		Liu F(2011)	179/445	411/235
		Mahmoud SM(2011)	138/333	401/461
		Sun S(2014)	31/73	45/59
		West NR(2013)	79/13	72/11
		Won KY(2013)	91/131	30/20
Her2 status(-/+)	8370	Aruga T(2009)	39/4	37/6
		Bates GJ(2006)	95/6	84/17
		Demir L(2013)	22/8	19/11
		Esther M. de Kruijf(2010)	213/27	157/15
		Ghebeh H(2008)	22/8	16/13
		Gobert M(2009)	109/8	54/7
		Liu F (2011)	481/143	424/222
		Liu S (2014)	1949/250	830/184
		Ma C (2012)	21/12	32/10

		Maeda N (2014)	34/13	16/27
		Mahmoud SM (2011)	473/24	731/151
		Seo AN (2013)	59/15	59/20
		Sun S (2014)	97/7	91/13
		Takenaka M (2013)	19/49	4/28
		West NR (2013)	62/24	58/18
		Won KY (2013)	168/54	38/12
		Yan M (2011)	238/7	186/20
Lymphatic metastasis(-/+)	6805	Bates GJ(2006)	74/41	53/57
		Esther M. de Kruijf(2010)	170/151	128/103
		Ghebeh H (2008)	5/14	15/26
		Gobert M (2009)	66/27	34/27
		Liu F(2011)	270/354	275/351
		Liu S(2014)	1311/930	545/484
		Ma C(2012)	12/24	30/15
		Maeda N(2014)	37/10	21/31
		Sun S(2014)	75/29	69/35
		West NR(2013)	29/51	37/40
		Won KY(2013)	130/92	31/19
		Yan M(2011)	154/107	109/107
Histological grade (I~II/III)	4453	Bates GJ(2006)	89/22	64/53
		Demir L(2013)	24/6	19/11
		Esther M. de Kruijf(2010)	215/100	140/97
		Ghebeh H(2008)	17/3	14/14
		Liu S(2014)	1111/1038	330/661
		Seo AN(2013)	34/40	44/35
		Won KY(2013)	165/57	21/29
Ki67(-/+)	990	Demir L(2013)	4/16	9/16
		Esther M. de Kruijf(2010)	270/30	176/44
		Seo AN(2013)	48/26	28/51
		Won KY	148/74	13/37
Distant metastasis(-/+)	1704	Mahmoud SM(2011)	372/145	617/298
		Won KY(2013)	220/2	50/0
P53 status(-/+)	314	Demir L(2013)	9/12	7/14

		Won KY(2013)	104/118	22/28
Vascular invasion(-/+)	4721	Ghebeh H(2008)	9/11	16/26
		Liu S(2014)	1198/962	522/461
		Maeda N(2014)	15/32	17/26
		Mahmoud SM(2011)	366/148	612/300
ER status(-/+)	8268	Aruga T (2009)	4/39	18/26
		Bates GJ (2006)	27/88	50/60
		Demir L (2013)	9/21	15/15
		Esther M. de Kruijf (2010)	109/191	88/135
		Ghebeh H (2008)	2/18	18/24
		Gobert M (2009)	25/103	22/38
		Liu F (2011)	118/506	374/252
		Liu S (2014)	521/1713	388/641
		Ma C (2012)	11/25	12/33
		Mahmoud SM (2011)	67/409	297/573
		Seo AN (2013)	16/58	37/42
		Sun S (2014)	19/85	28/48
		Takenaka M (2013)	31/37	25/7
		Won KY (2013)	72/150	31/19
		Yan M (2011)	73/189	81/135